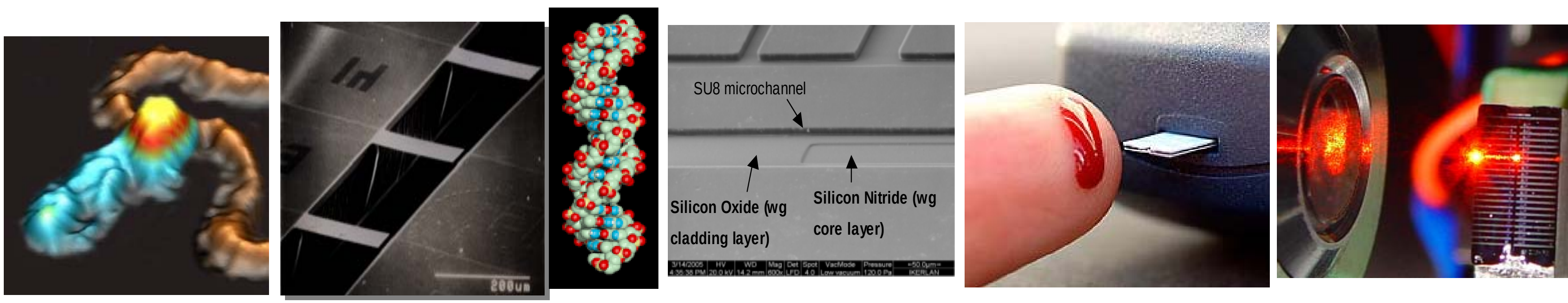
“Lab-on-a-chip” nanobiosensor platforms for real-time and early diagnostic of diseases



Prof. Laura M. Lechuga

Nanobiosensors and Molecular Nanobiophysics Group
Reseach Center on Nanoscience and Nanotechnology **(CIN2: CSIC-ICN)**
CIBER on Bioengineering, Biomaterials and Nanomedicine **(CIBER-BBN)**
Barcelona (Spain)

Research Centre on Nanoscience and Nanotechnology

CIN2 (CSIC-ICN)

(Campus UAB, Barcelona, Spain)

- Building will be ready in summer 2009. Fully running at the end´09
- 13 Research Groups running, more than 150 people. Centre for 250 persons. 6000 m^{2}.
- Acess to the largest Clean Room in Spain (CNM-CSIC) with nanotech area.

MULTIDISCIPLINARY RESEARCH

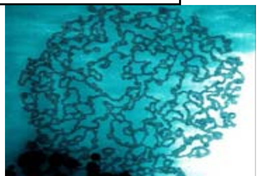
- **Nanoionics, nanooptics, nanomagnetism**
- **Synthesis of nanoparticles**
- **Surface nanostructuring**
- **Nanoestructured hybrid materials**
- **Transport in nanoentities (CNT)**
- **Nanobiosensors, Nanobiophysics**
- **Nanotechnology for energy**
- **Toxicity of Nanotechnology**
- **Common research with ALBA synchrotron**

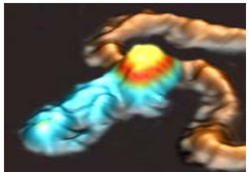
TECHNOLOGICAL TRANSFER

SENSIA, SL

ENDOR, SL

More information in www.cin2.es





ICN Institut Català de Nanotecnologia

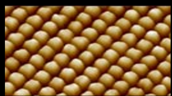
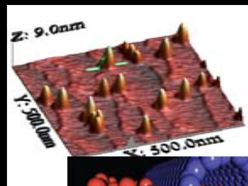
CSIC

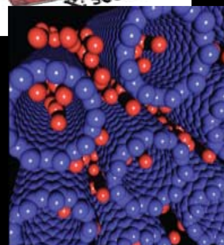
Outline

- Introduction: Nanomedicine, Nanodiagnostics, Biosensors
- Nanobiosensors for diagnostics
  - Plasmonic sensors
  - Integrated Nanophotonic sensors
  - Nanomechanical sensors
- Lab-on-a-chip microsystems
- Applications: Early detection of cancer
- Future perspectives

PROSPECTS IN NANOTECHNOLOGY

MATERIALS

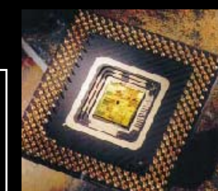
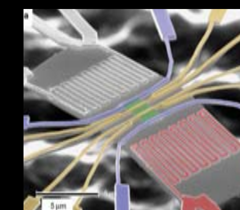



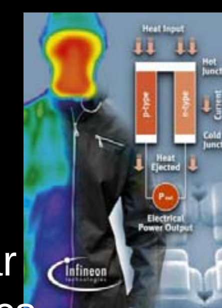


CNTs, NPs
Nanowires
Nanoparticles
Graphene
Nanocomposites
Qdots




ELECTRONICS



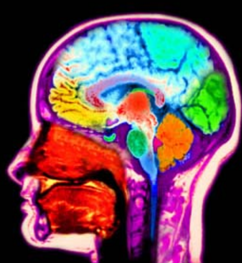
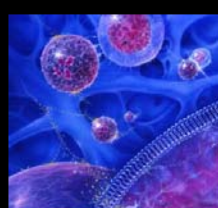
Molecular electronics

Nanochips

Wereable electronics

MP3, GPS

MEDICINE

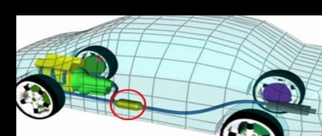
ENERGY

Fuel cells

Solar cells

Bateries

Ultracapacitors

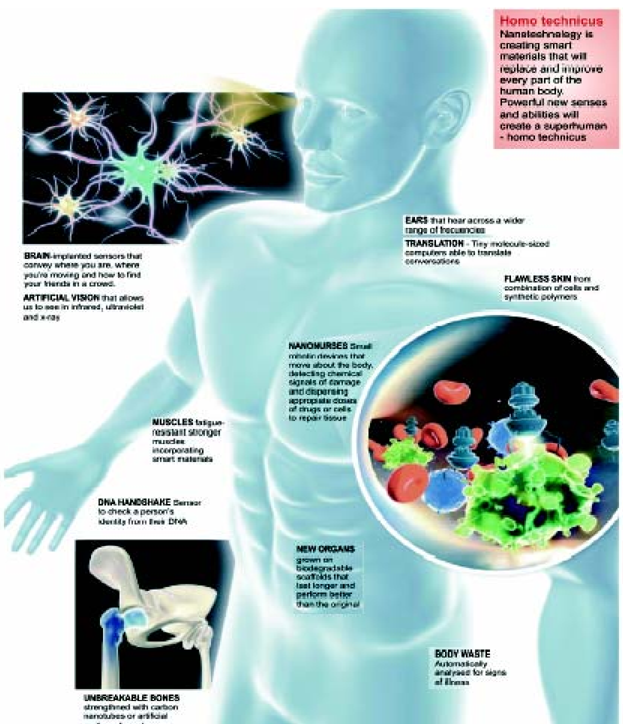



What is Nanomedicine?

Application of materials, devices and process from Nanotechnology to develop nano-sized tools for the diagnosis, prevention and treatment of diseases, mainly at the initial stage.

Nanomedicine

- **Nanodiagnostics:** Nanosensors, imaging
- **Targeted Drug Delivery**
- **Regenerative Medicine:** Gene Therapy, Cell Therapy, Tissue Engineering

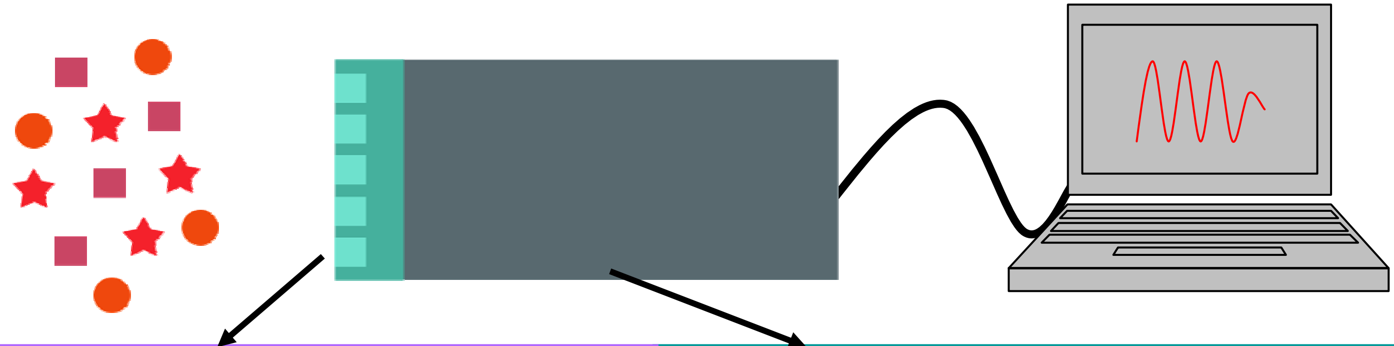


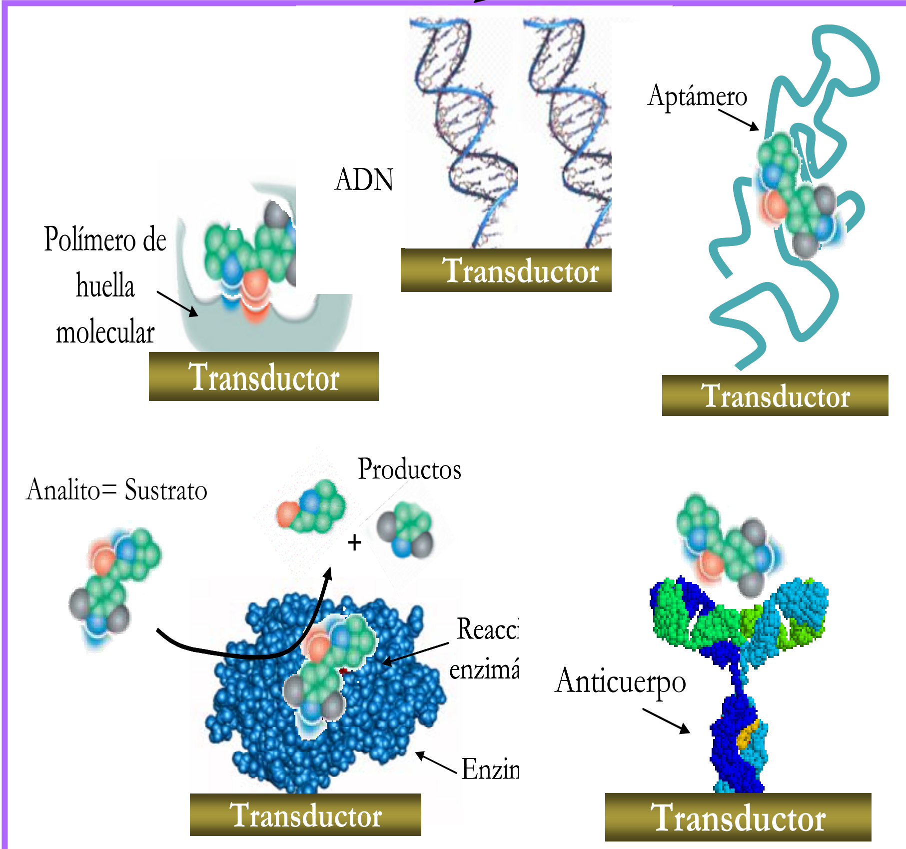
<u>To improve the prevention, diagnosis and therapy in Human Health</u>

"Find, fight and follow"

The ultimate goal is to improve the quality of life

BIOSENSOR DEVICE





ELECTROCHEMICAL
- amperométricos
- potenciométricos
- conductométricos

✓Sensibles
✓ Miniaturización y multiplexado
✕ Mediadores para amplificar la señal

OPTICAL
- absorption
- fluorescence
- **evanescent wave**

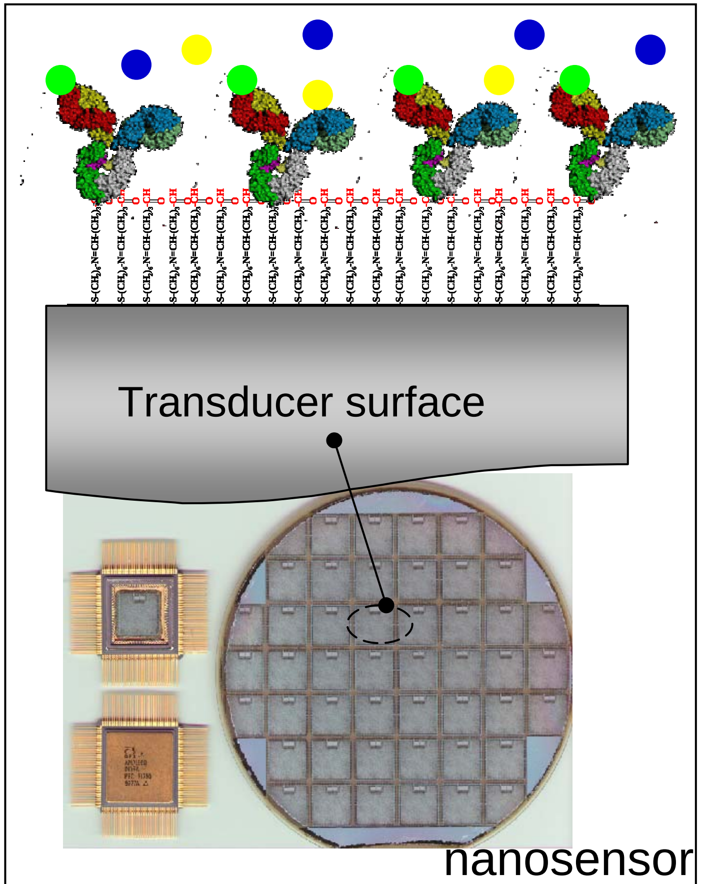
✓ Altamente sensible
✓ Técnica robusta y reproducible
✓ Alta capacidad de multiplexado y miniaturización

MECHANICAL
- piezoelectric
- **nanomechanical**

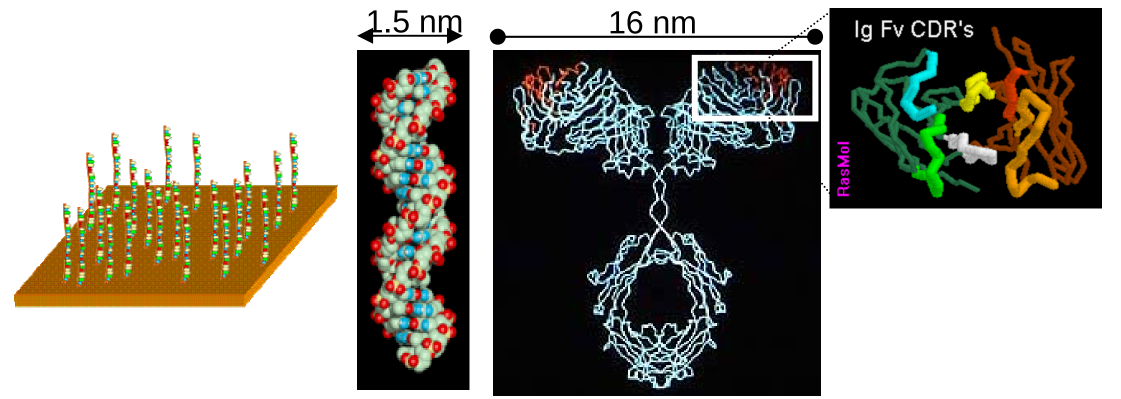
✓ Sensibles
✓ Miniaturización y multiplexado
✕ Principio de funcionamiento poco establecido

NANOBIOSENSORS

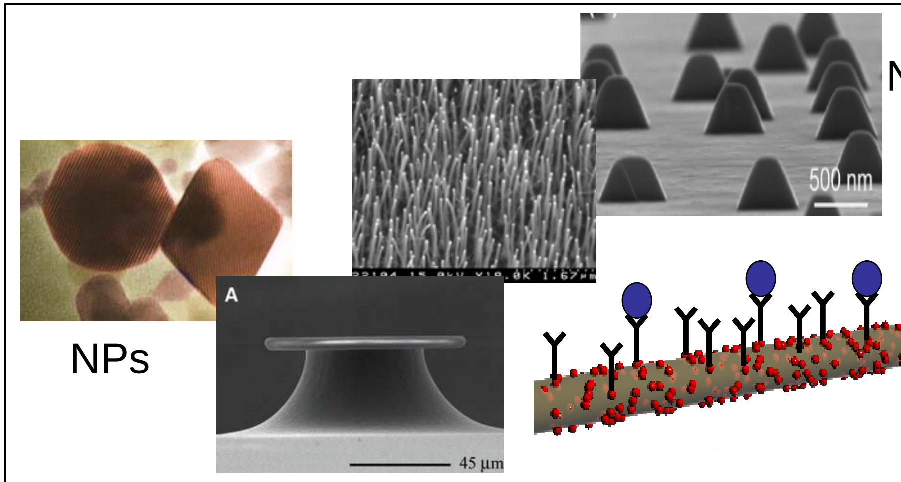
The key is the bioengineering of the sensor interface



Nanobiostructures



Oriented, stable, optimal surface densidad superficial óptima, no adsorciones inespecíficas



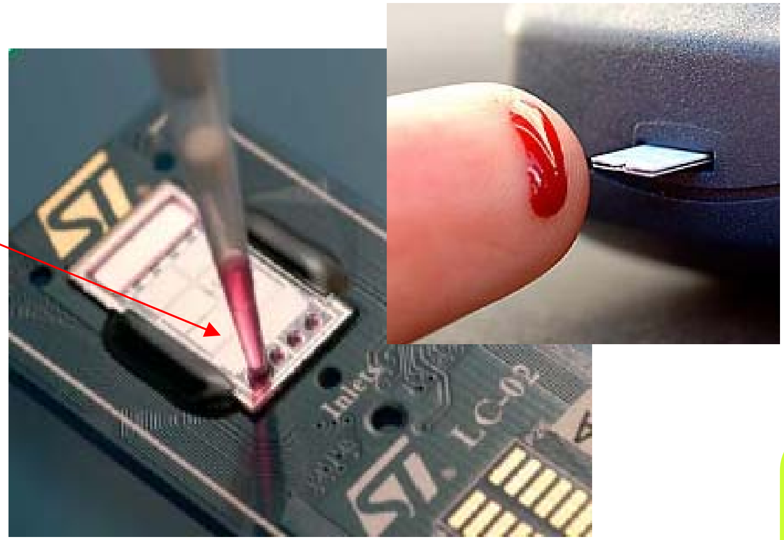
Nanoarrays

NPs

CNTs

CENTRE D'INVESTIGACIÓ
EN NANOCIÈNCIA I
NANOTECNOLOGIA
CAMPUS UAB. BELLATERRA. BARCELONA

"Lab-on-a-chip" nanobiosensor microsystem



- Nanobiosensores
- Microfluidica
- Electrónica y procesado datos
- fuentes y detectores

"POCT" point-of care testing

- precision
- sensitivity
- selectivity
- label-free
- fast
- stability
- no pre-treatment

- Instant Diagnostic
- In any place at any time
- Can operate inside the human body

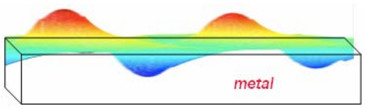
Some applications	Aim
Drug development	Targeted and rapid design of new active ingredients
Cancer research	Identification of cancer genes and individual
Microbiology	Classification of microorganisms
Food industry	Analysis of various disease pathogens in food

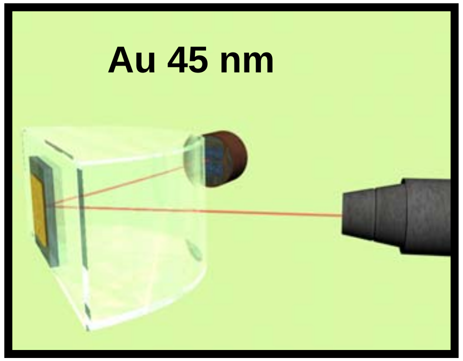
PLASMONIC

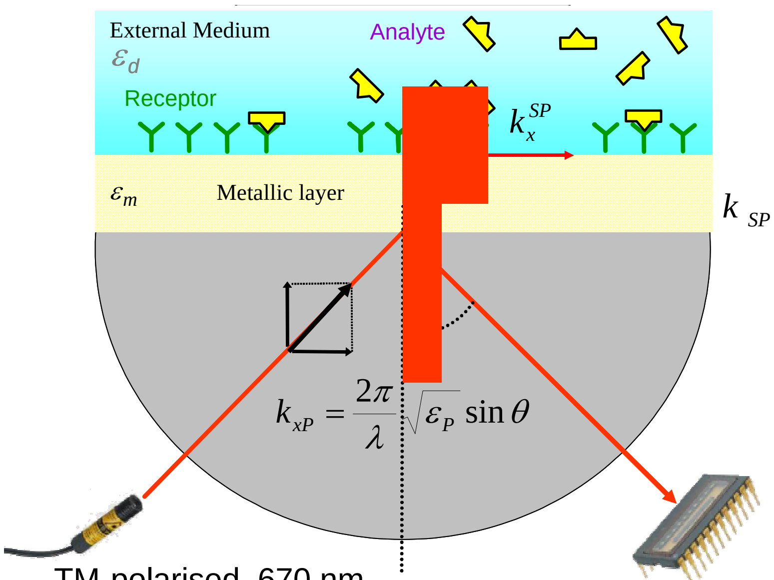
NANOBIOSENSORS

Surface Plasmon Resonance Biosensor (SPR)

SPR working principle

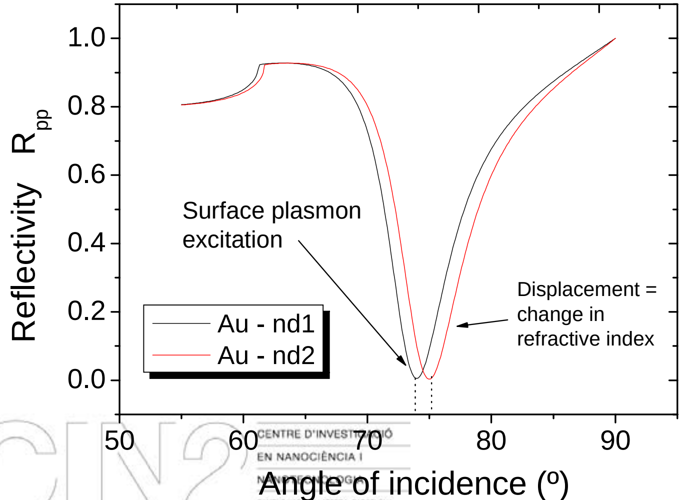


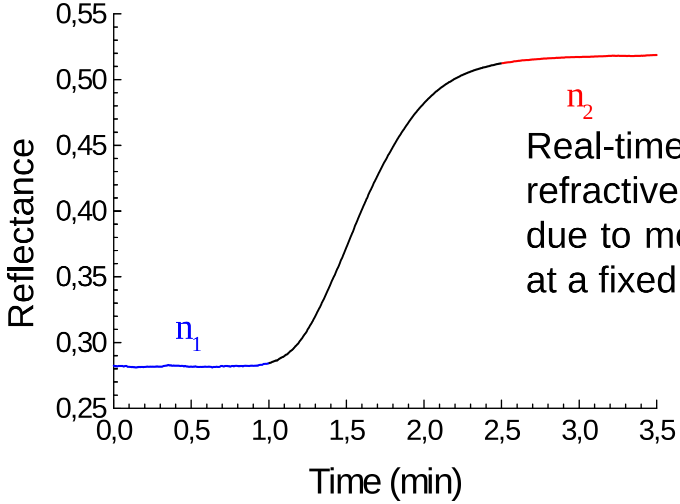




$$k_{SP} = k_0 \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_d + \varepsilon_m}}$$

Determination of refractive index changes as a variation of the Resonance angle



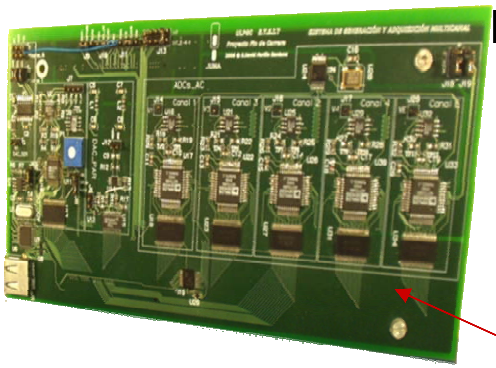


$\Delta n_{min} \sim 10^{-5}$

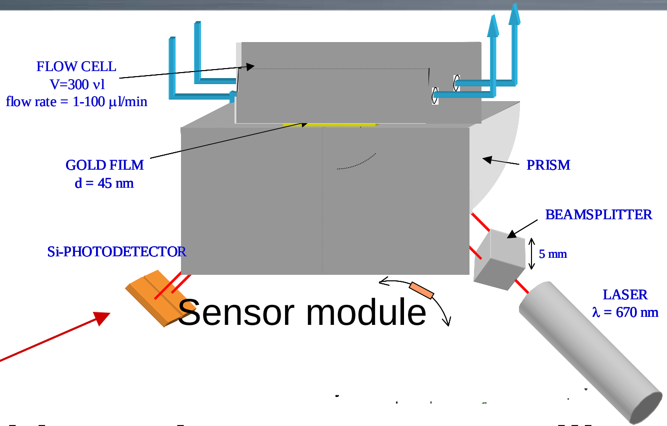
Real-time detection of refractive index variations due to molecular interactions at a fixed angle

Plasmonic Biosensor (SPR)

Portable device, two channels

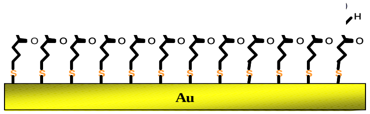


electronics

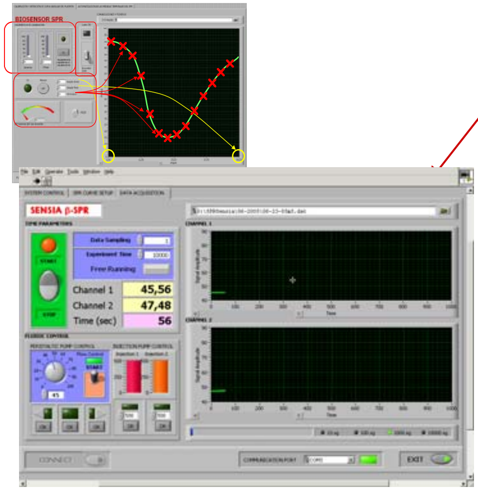


Sensor module

(old version, new one will be release at the end´08)



Immobilization and regeneration



Software

Wireless transmission module

Flow delivery System

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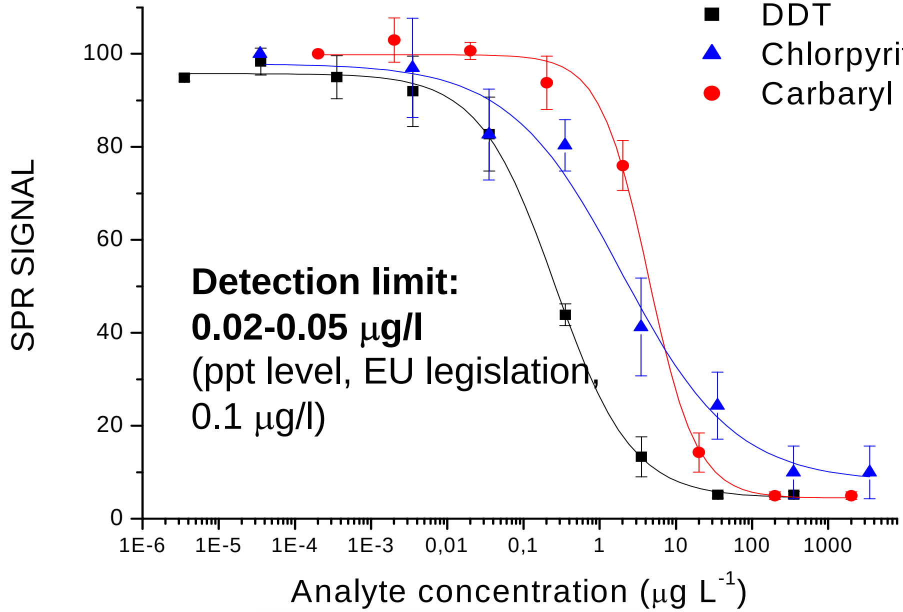
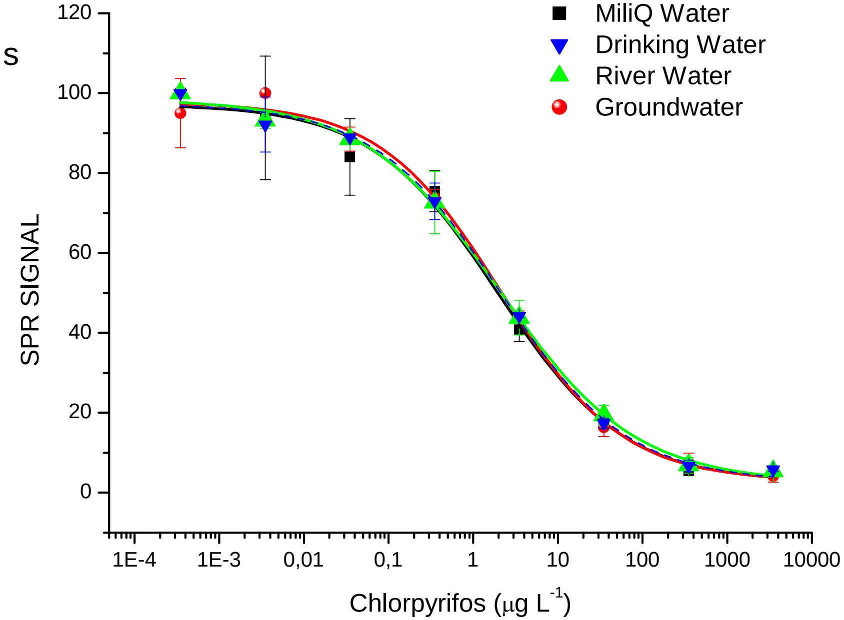
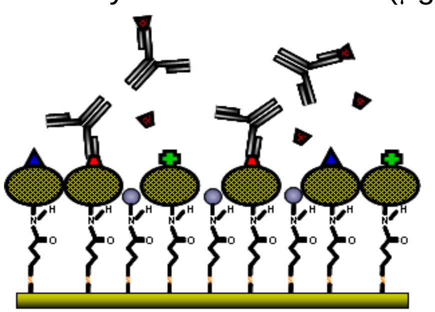
SPR Biosensor: applications

Environmental toxic pollutants in real samples (water safety)

INHIBITION IMMUNOASSAY





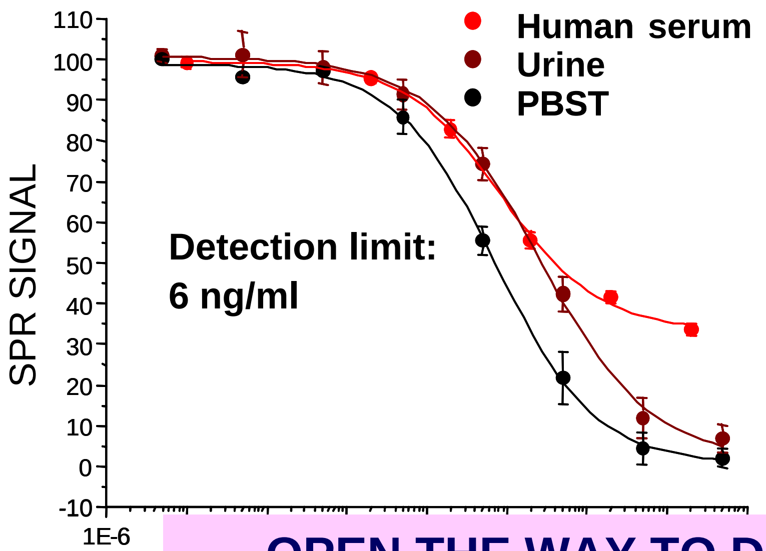


- **Direct detection in real water samples**
- **Monoclonal antibodies**
- **No pre-treatment, fast**
- **Cycle of analysis 20 min**
- **More than 200 regenerable**
- **Validated results**
- **up to 6 analytes same analysis!!**

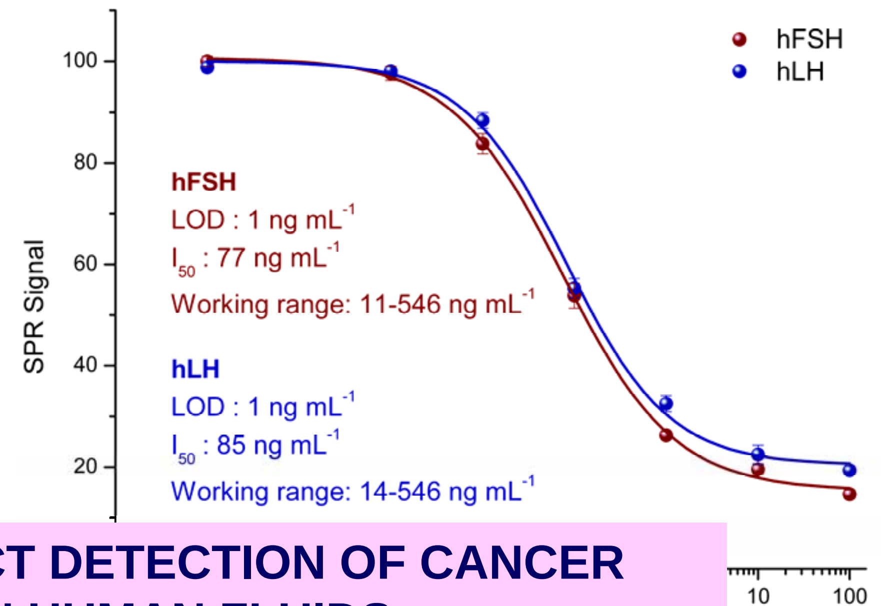
Anal. Bioanal. Chem. 387, 1449 (2007)
Biosens.& Bioelec. 22, 1410 (2007)
Anal. Bioanal. Chem. 388, 207 (2007)
Sens.Actu. B 118, 399 (2006)
Talanta 69 (2), 359 (2006)
Anal. Chim. Acta 561, 40 (2006)
Biosens.& Bioelec.21, 2129 (2006)

SPR Biosensor: Clinical diagnosis in human samples (urine, serum)

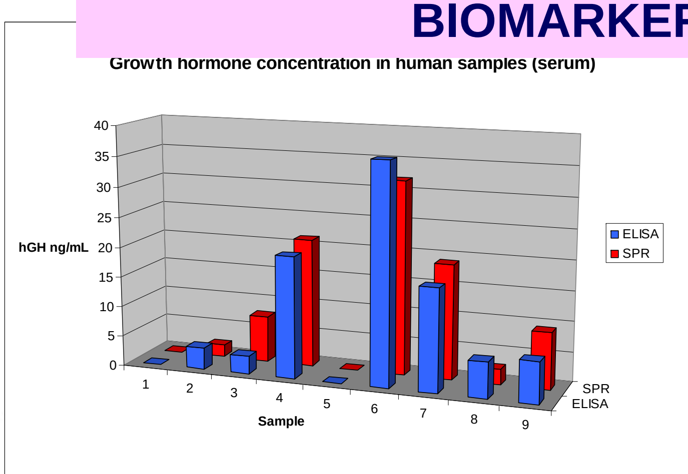
Human Growth hormone



Luteinizing hormone (hLH), Folicle stimulating hormone (FSH)



OPEN THE WAY TO DIRECT DETECTION OF CANCER BIOMARKERS IN HUMAN FLUIDS



- Direct detection in real human samples
- No pre-treatment
- Detection at physiological levels
- More than 100 regenerable
- Validated results
- Fast
- Low sample volume

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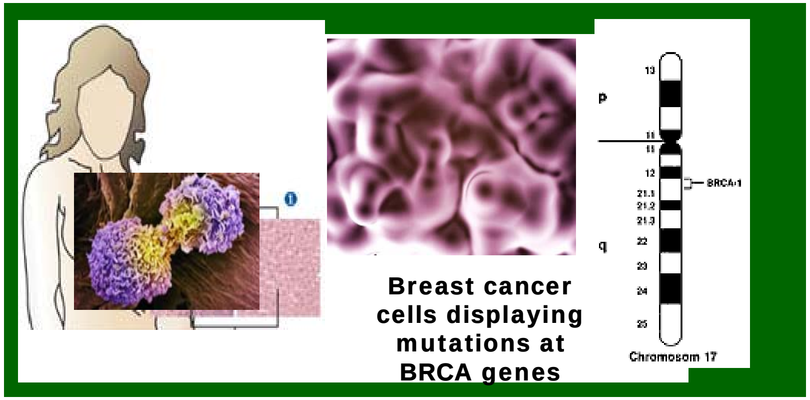
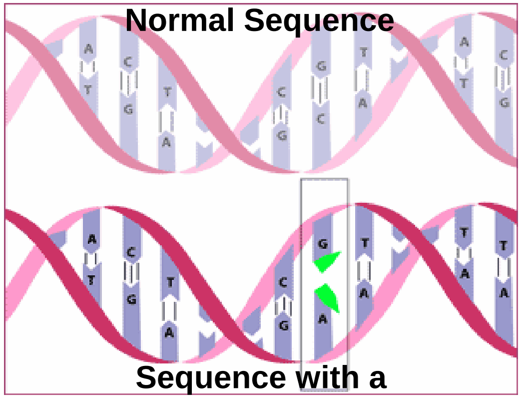
CSIC

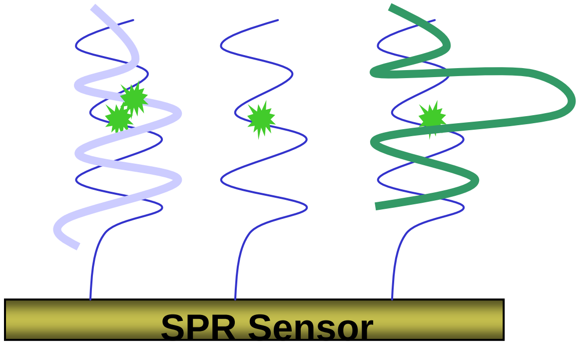
SPR Biosensor: Early cancer diagnostics

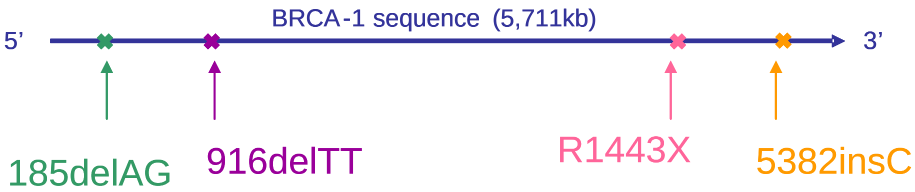
EARLY DETECTION OF INHERITED BREAST CANCER

Detection of point mutations at BRCA-1

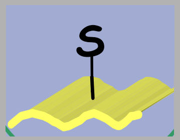








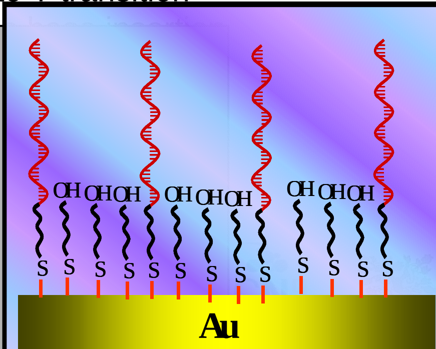
185delAG = two bases (AG) deletion
916delTT = two bases (TT) deletion
R1443X = C to T transition



Covalent attachment of thiol-derivatized DNA probes

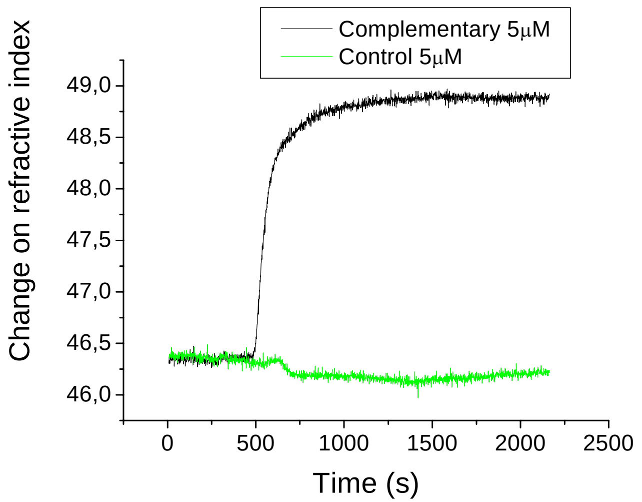
DNA self-assembled monolayers

(Optimisation of buffer concentration, stringency conditions, aditives, lateral and vertical spacers, Tª,)

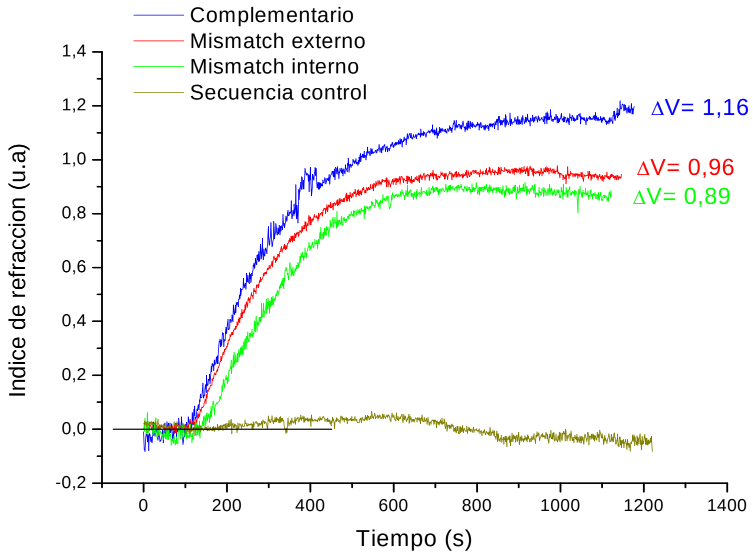


SPR Biosensor: Early cancer diagnostics

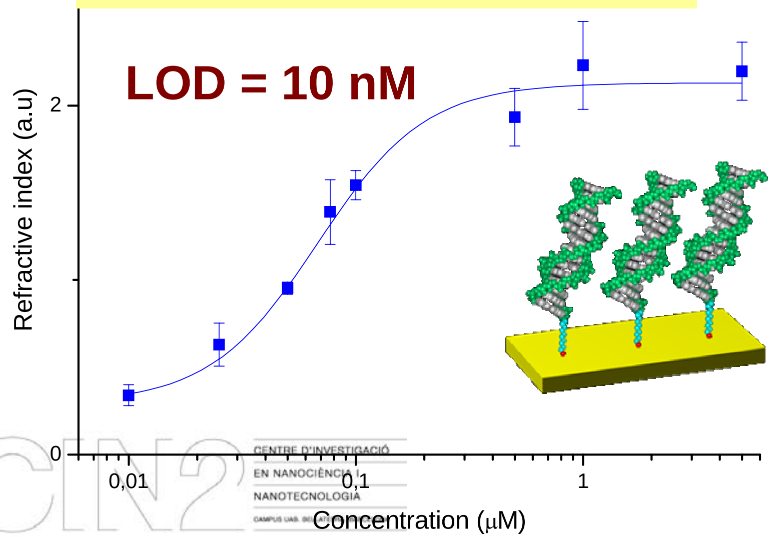
Hybridisation detection



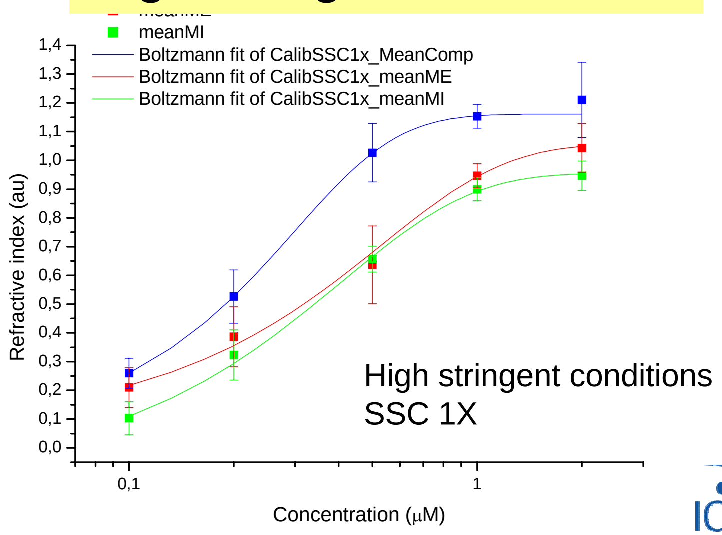
Detection of mutations



Non stringent conditions



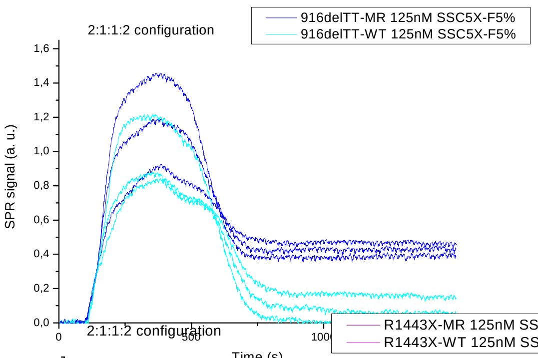
High stringent conditions

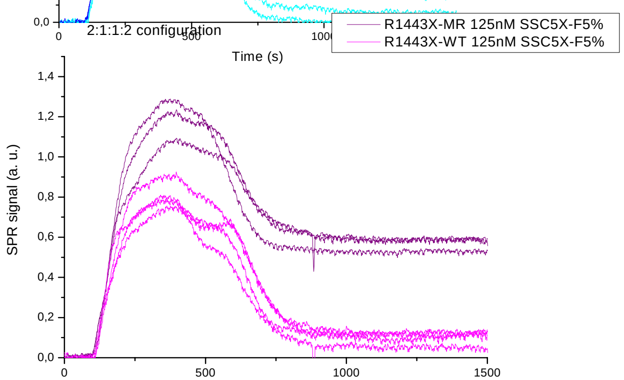


Hybridization with PCR oligos-like: Tetra-analyte detection format

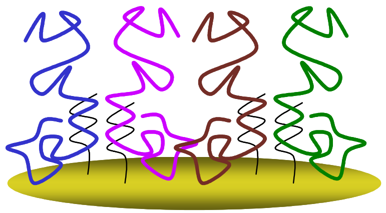
185delAG = two bases (AG) deletion
916delTT = two bases (TT) deletion

R1443X = C to T transition
5382insC = one base (C) insertion



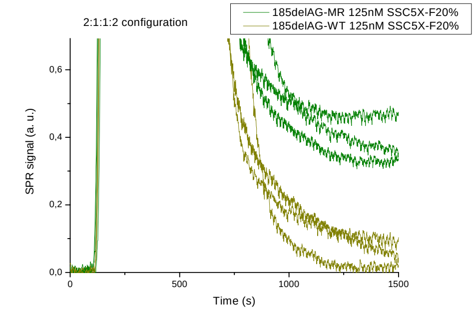


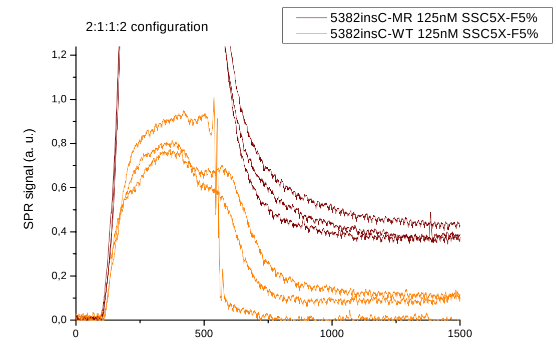
Gene BRCA-1



Sensor chip

Time scale: less than 1 hour

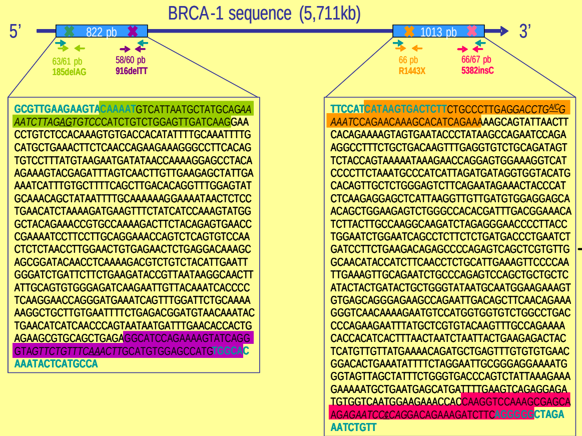




Patient

DNA Extraction

PCR



To the sensor for analysis

BUT with SPR biosensors..........

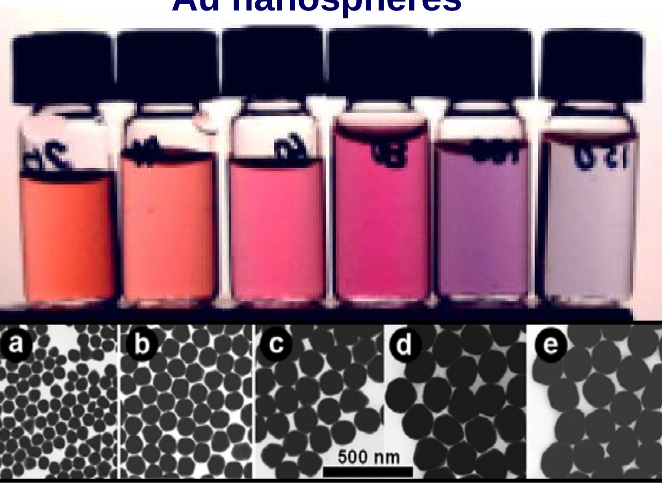
- No sensitivity for small analytes and very low concentrations (pM-femtoM, single-molecule detection)
- Although portable device, size and weight are not optimal
- Multianalyte capabilities for high throughput

Higher sensitivity is required
Microsystems platforms
High throughput/multiplexing capabilities

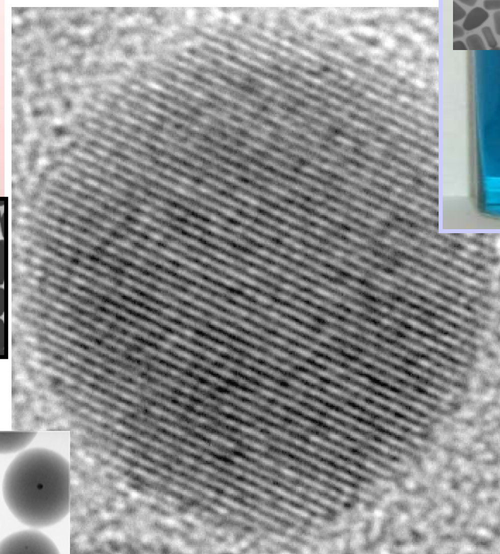
1) **Integrated photonic Mach-Zehnder nanointerferometers**
2) **Nanomechanical biosensors (standard and optical microcantilevers)**
3) **Localised Surface Plasmon Resonance in nanoparticles (LSPR) and magnetoplasmonic biosensors**

Localised plasmon in Nanoparticles

Au nanospheres

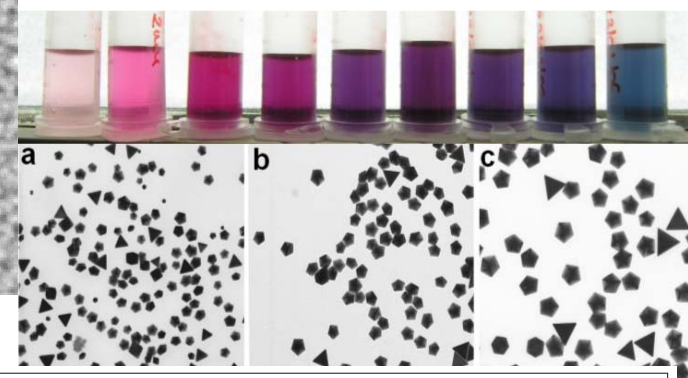


EM strongly localized

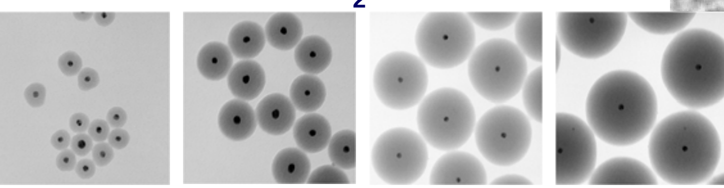


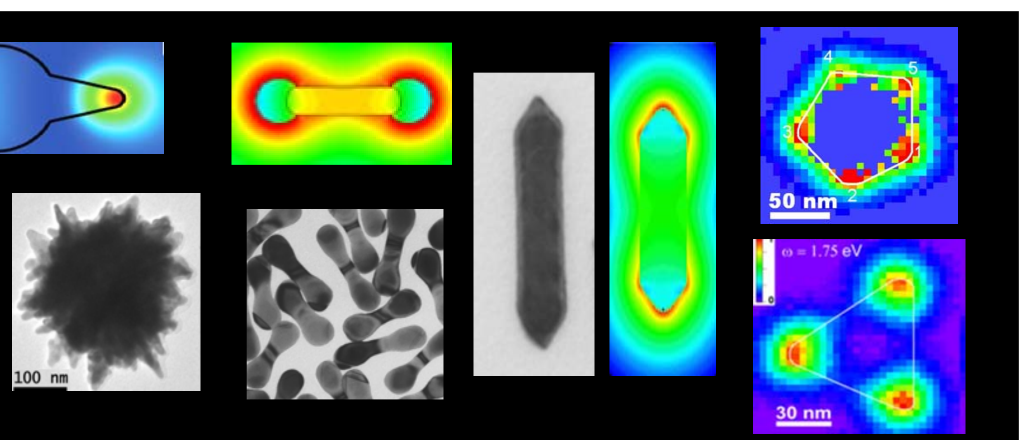
Au nanorods

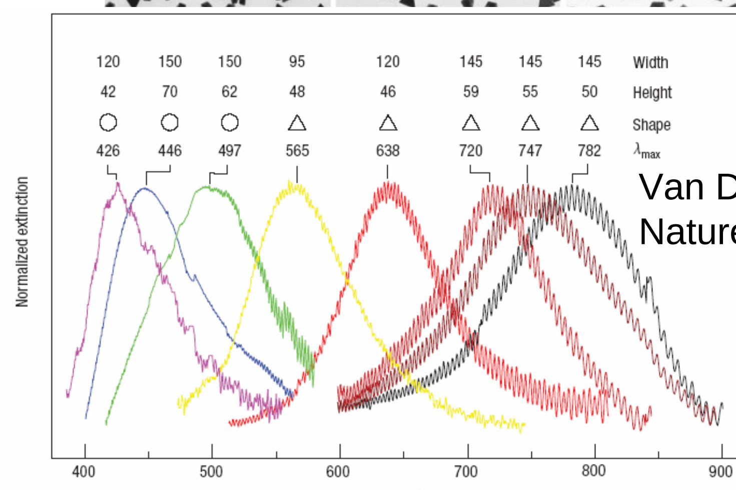
Au decahedra



Au/SiO_2



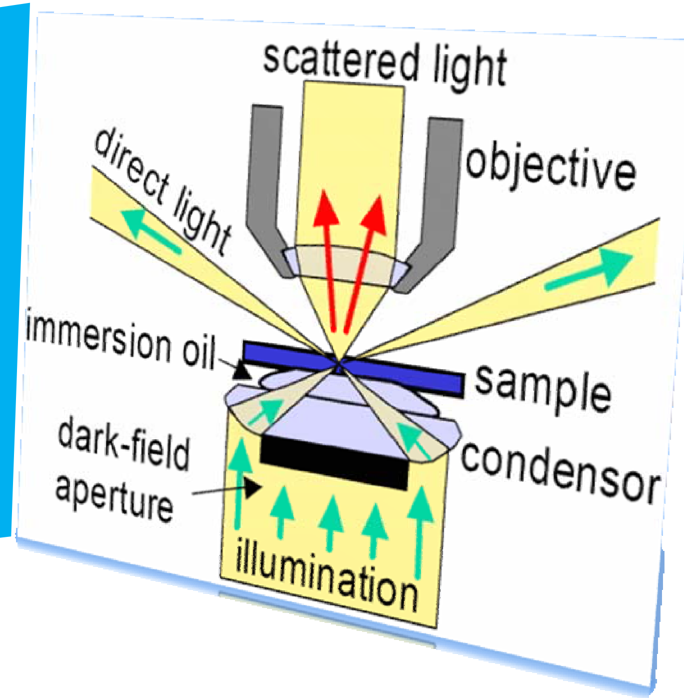




Van Duyne, Nature 2008

Prof. Luis Liz- Marzán, Univ. Vigo

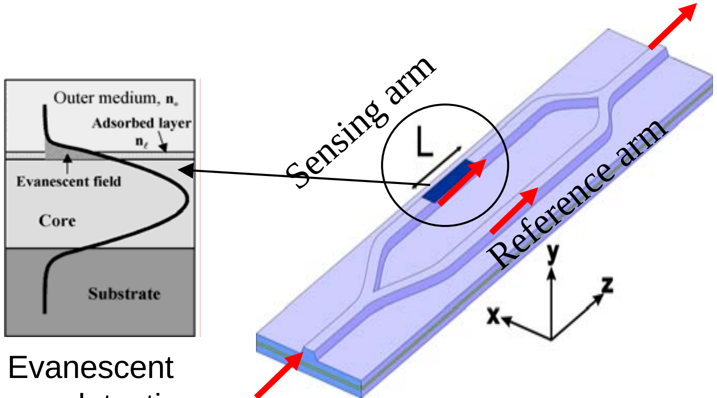
LSPR: Dark-field Microscopy



OPEN THE POSSIBILITY OF HIGHLY DENSE NANOBIOSENSING ARRAYS FOR THOUSANDS OF ANALYTES

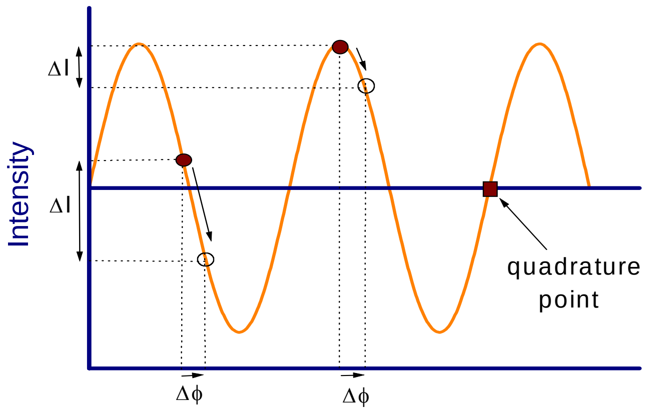
PHOTONIC NANOBIOSENSORS

Photonic Nanosensor: Mach-Zehnder Interferometer



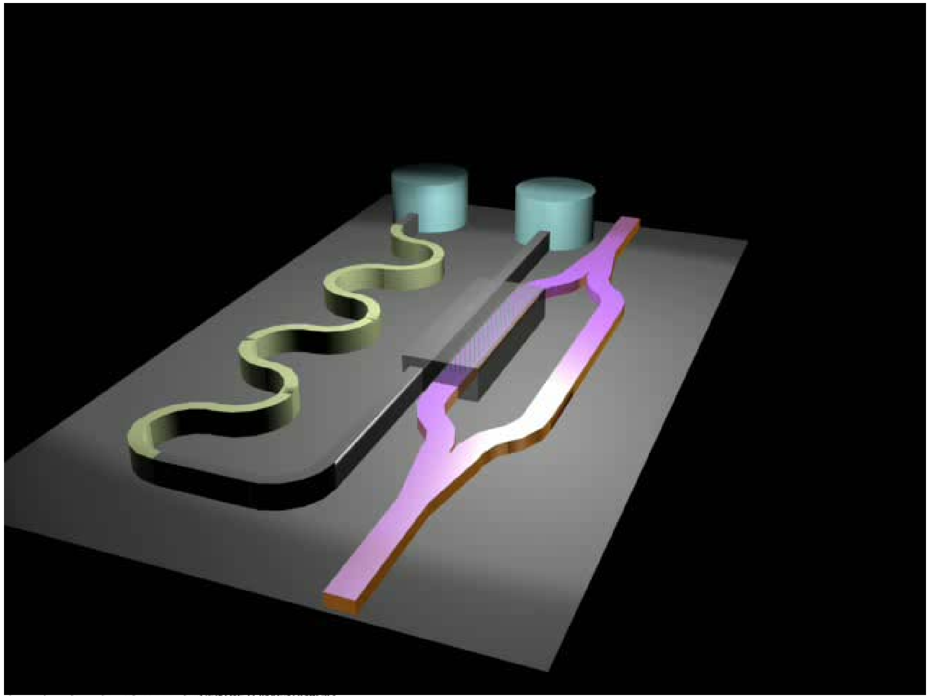
Evanescent wave detection

Y-junctions: 50% splitting and recombining



$$I = I_o \cdot \delta^2 \cdot [1 + V \cdot cos(\Delta\Phi)]$$

$$\Delta\Phi = \frac{2\pi}{\lambda} \cdot \Delta N \cdot L$$

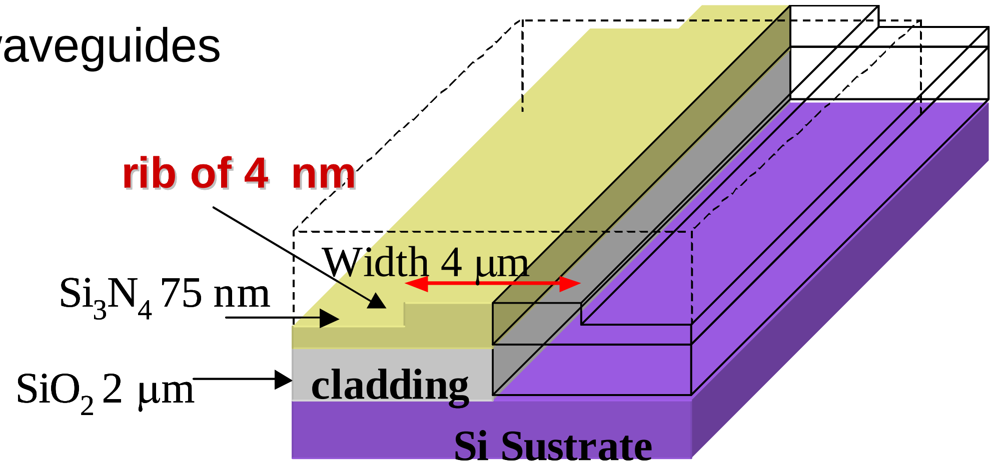


Design of the system

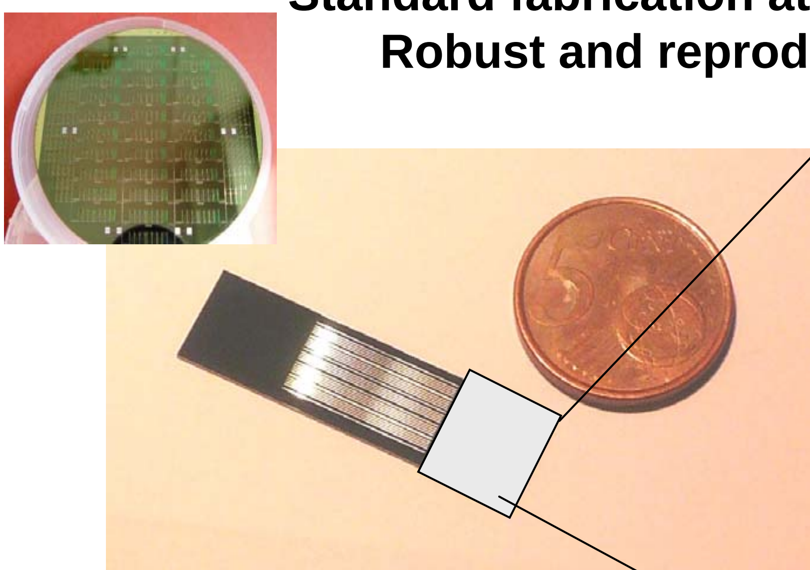
- *Single mode behaviour*
- *High surface sensitivity*

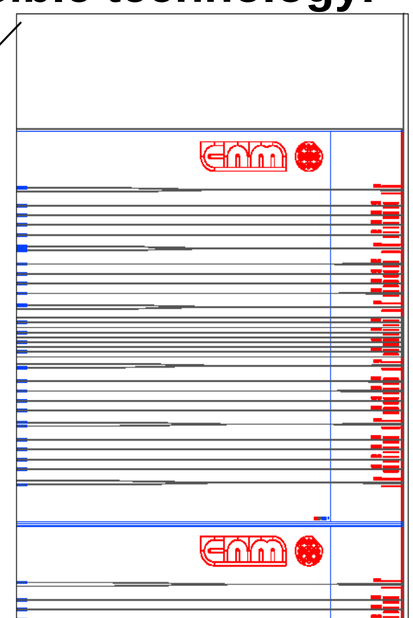
Photonic chip

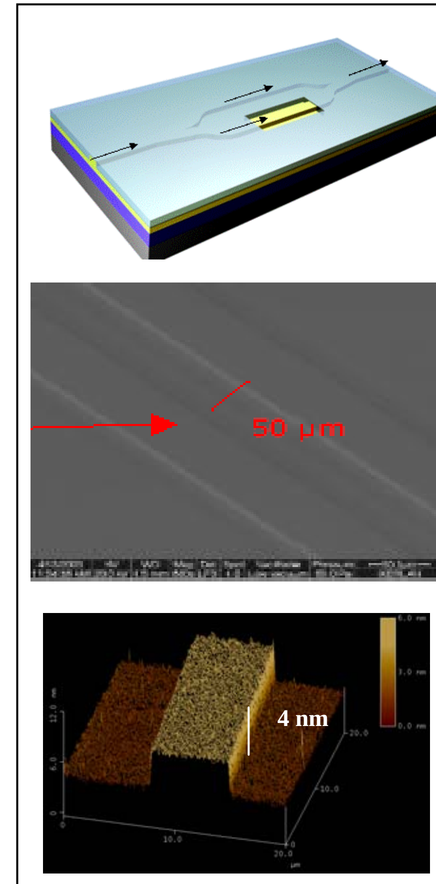
Optical waveguides



Standard fabrication at Clean Room facilities.
Robust and reproducible technology.



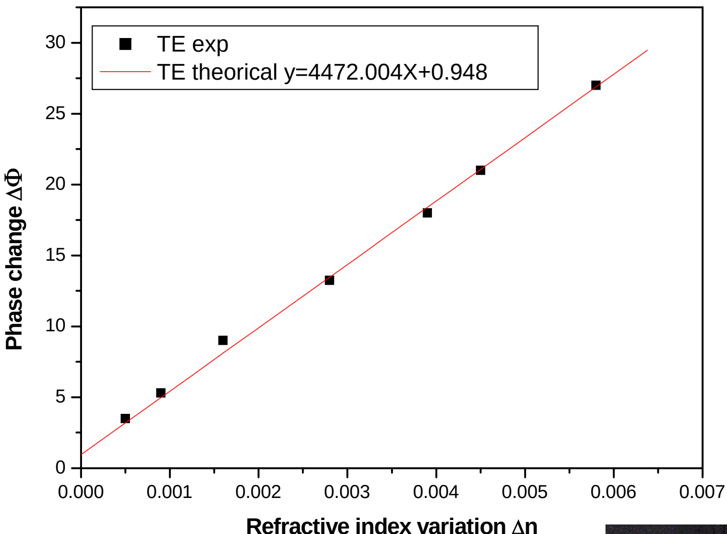




Advances in Optical Technologies ID 383927, 2008

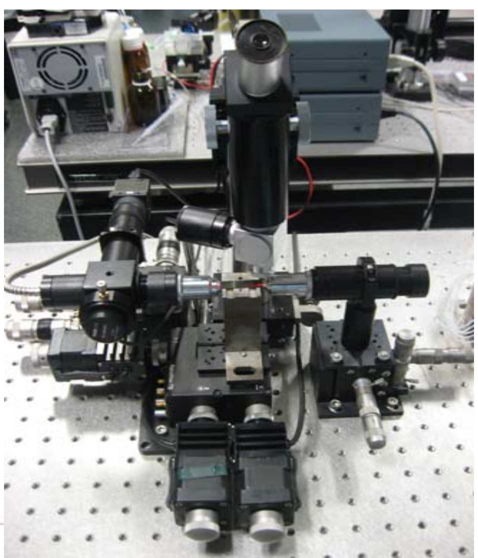
MZI Performance

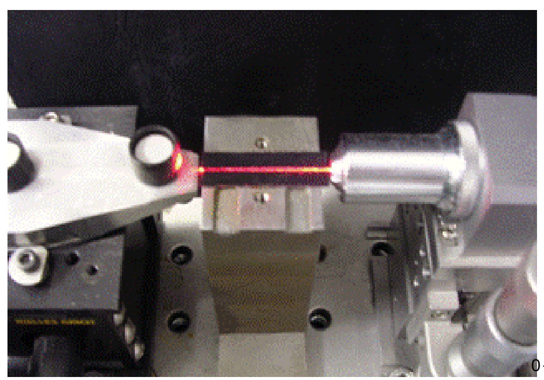
Sensitivity evaluation

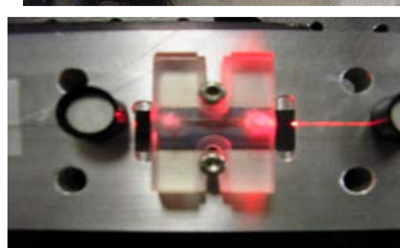


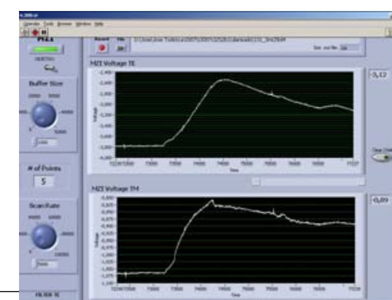
$\Delta n_{o,min}$ = **1.1x10^{-7}** $\Delta N_{eff,\,min}$ = **2.0x10^{-8}**

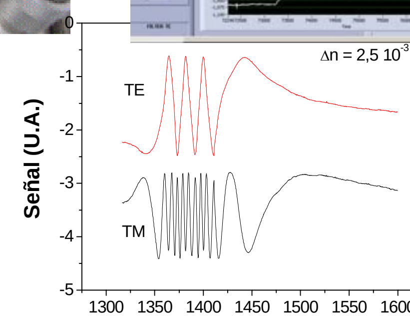
The detection limit value corresponds to an average growth layer of 1.10^{-4} nm (3.10^{5} mol.cm^{2}) and a Surface Sensitivity around 2.10^{-4} nm^{-1}. Direct detection <u>in the picomolar range</u> (10^{-12} M) is possible (60 fg/ mm^{2})

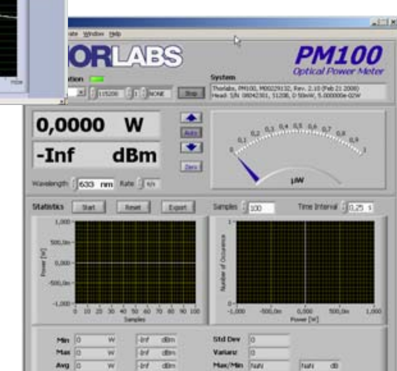








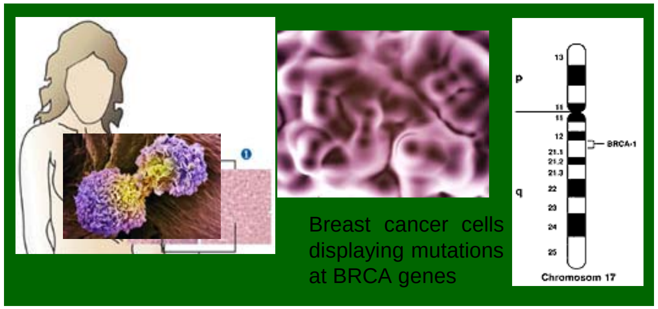


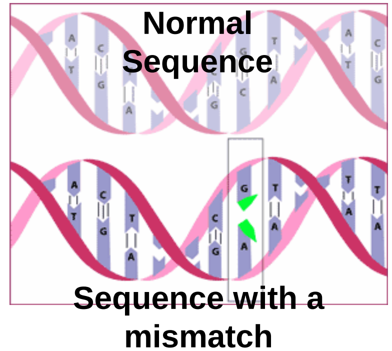


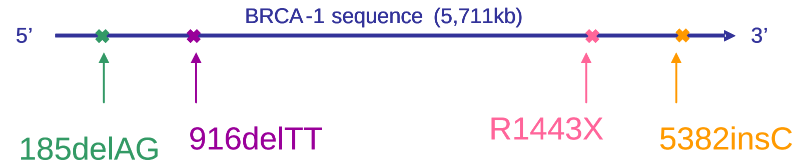
MZI Sensor: Clinical diagnosis

EARLY DETECTION OF INHERITED BREAST CANCER

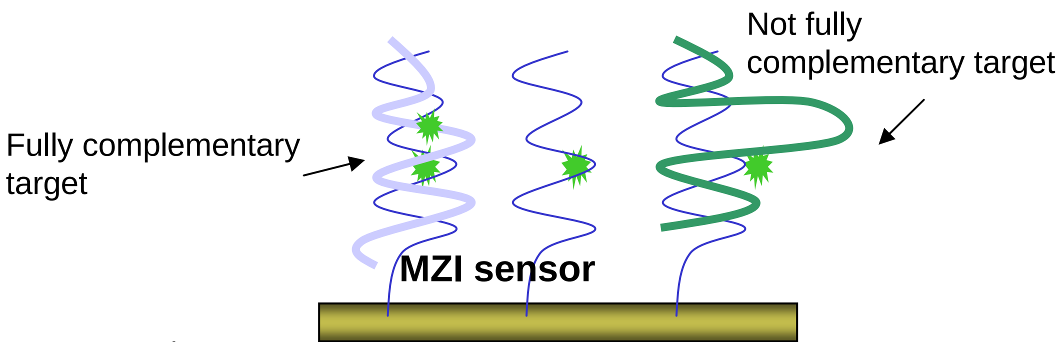
Detection of point mutations at BRCA-1







185delAG = two bases (AG) deletion
916delTT = two bases (TT) deletion
R1443X = C to T transition
5382insC = one base insertion

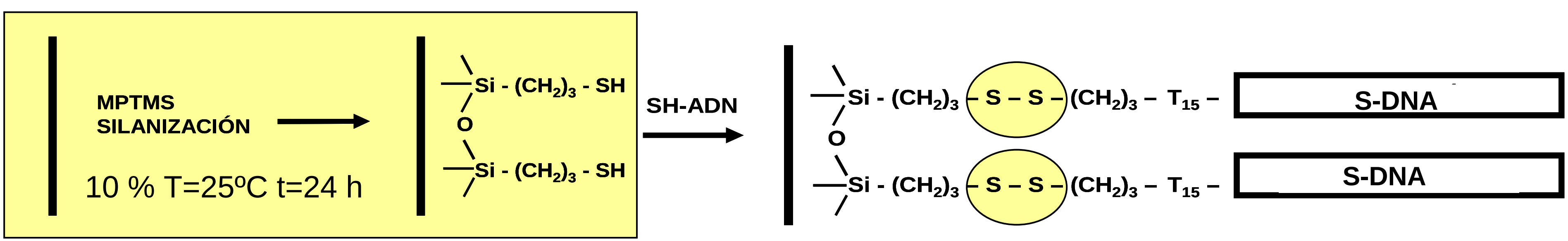


BRCA-1 Gene

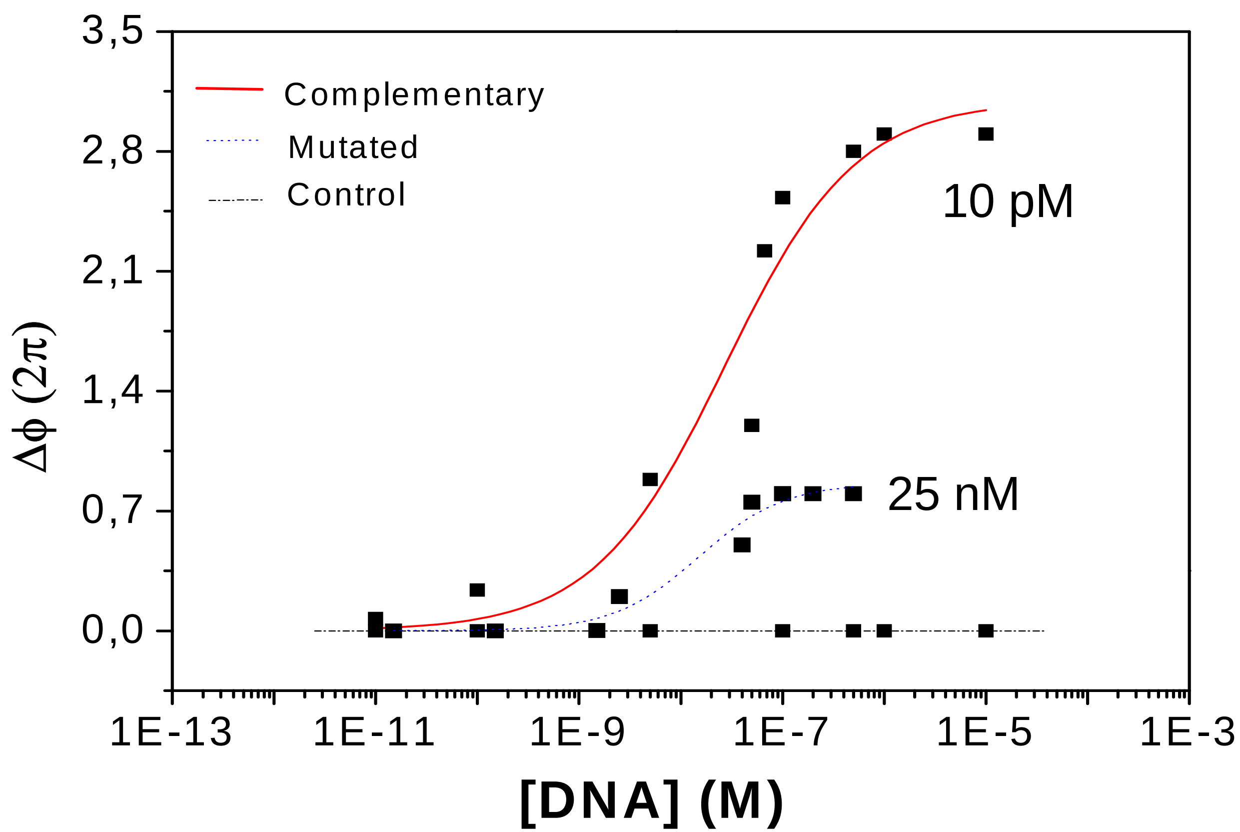
DNA probe (28 mer):5'-SH-$(CH_2)_6$-(15T)-**GTT CTG TCA AAC** T-3´

DNA target (58 mer): 5' – TGC CAC ATG GCT CCA CAT GCA **AGT TTG ACA GAA** CTA CCC TGA TTT TCT GCA C – 3'

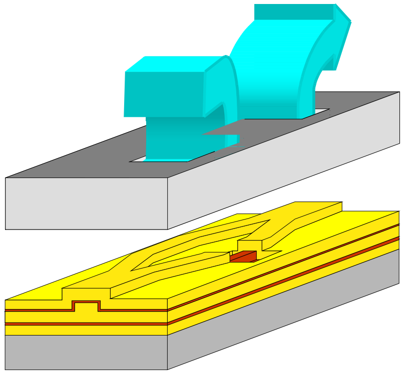
DNA (mutations):5´- TGC CAC ATG GCT CCA CAT GCA **AGT TTG AAA CA GAAC**TAC CCT GAT ACT TTT CTG GAT GCC -3´



Nanophotonic chip: Results

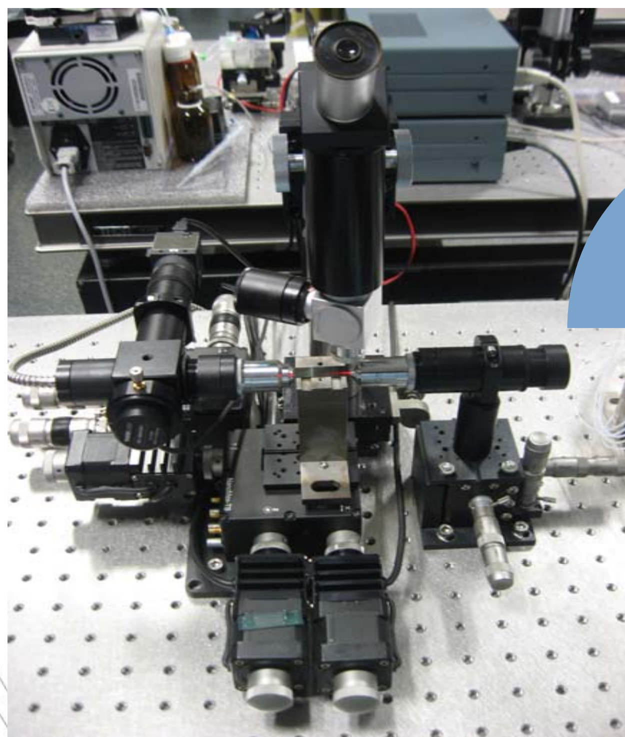


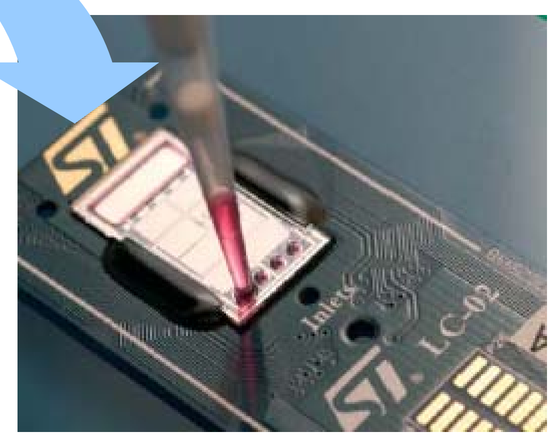
From *chip on a lab* to “*lab on a chip*”



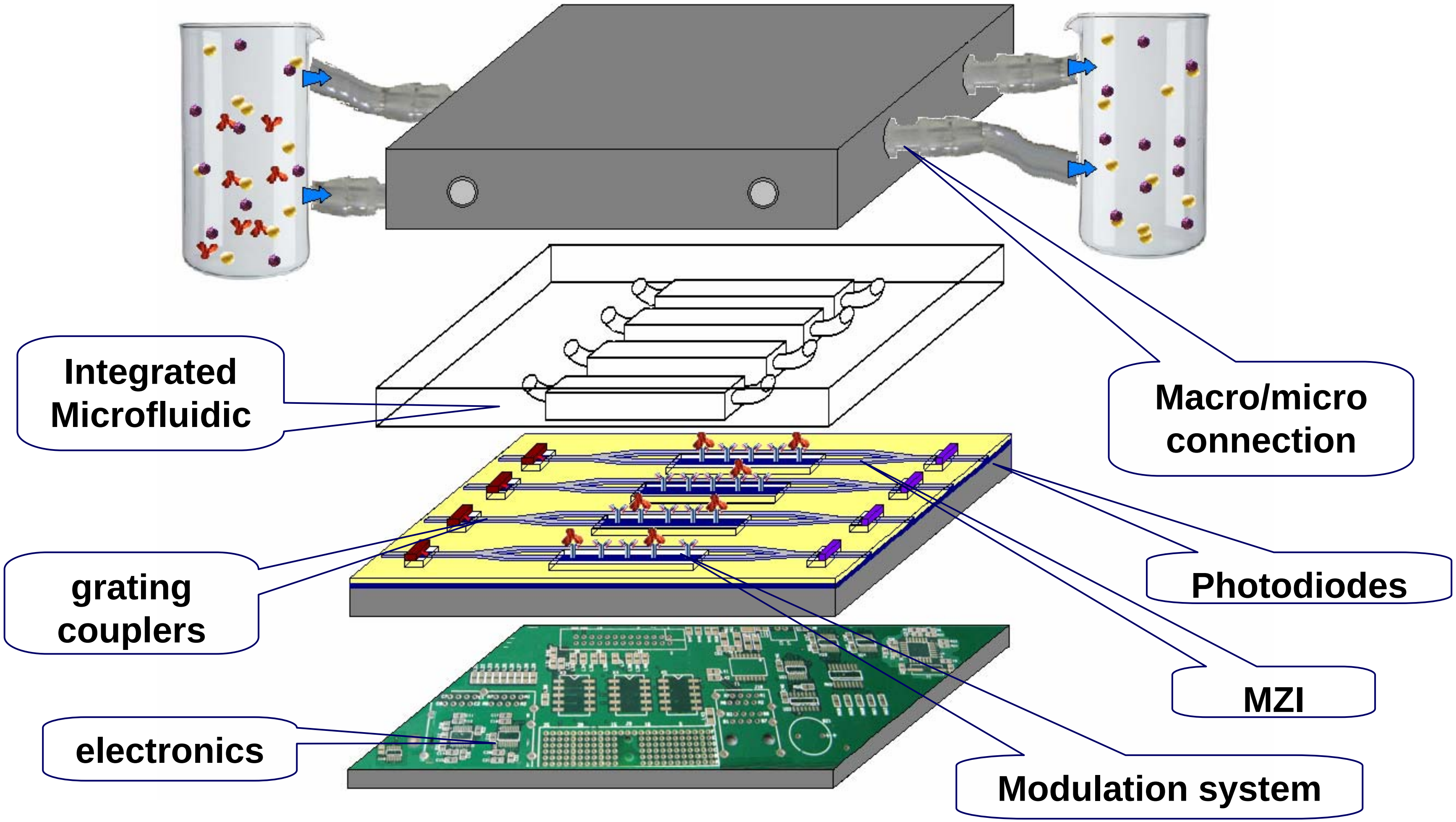
Results using a macro-flow cell: only one sensor, high volume of reagents and samples.

Integration of micro/nanobiosensors in platforms with microfluidics, biological reagents, excitation and signal acquisition and processing.



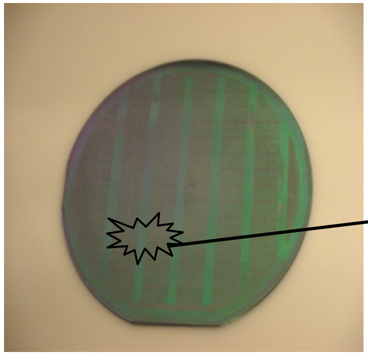


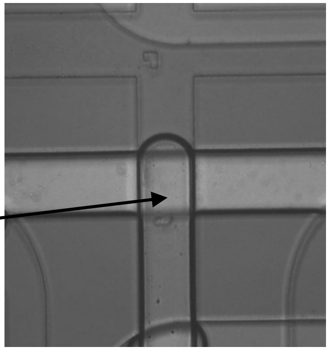
"Lab-on-a-chip" Biosensor microsystem: Our approach

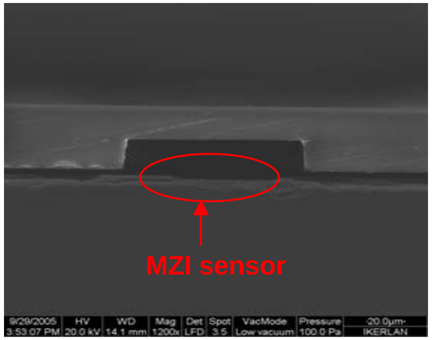


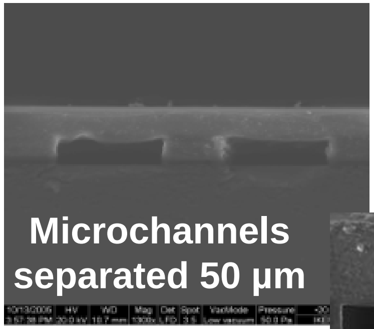
Fully integration of optical functions within microfluidic chips is in its infancy
on-chip approach

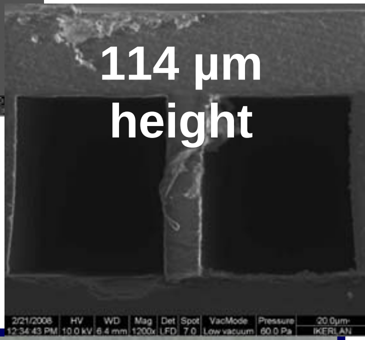
Nanosensor/microfluidics integration



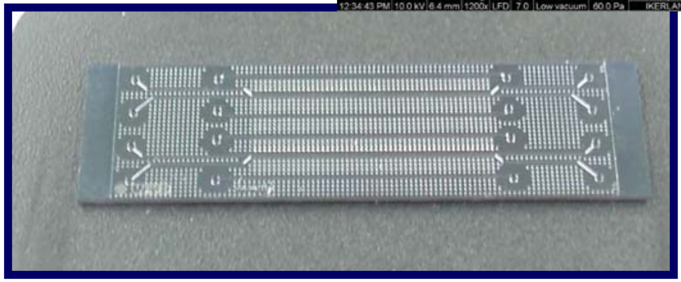


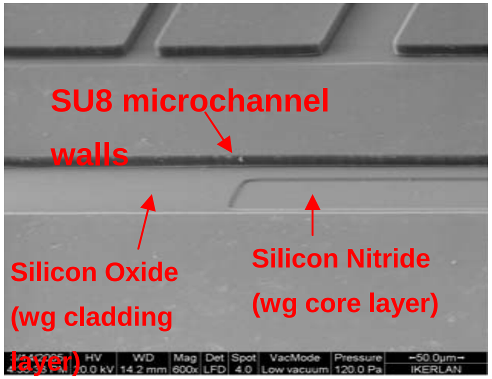




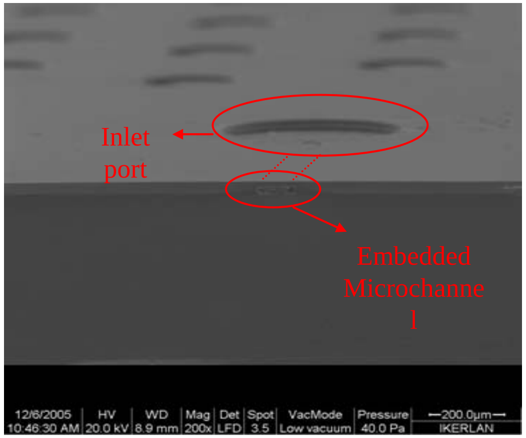


Channels of 20-115 µm height and 50-150 µm width

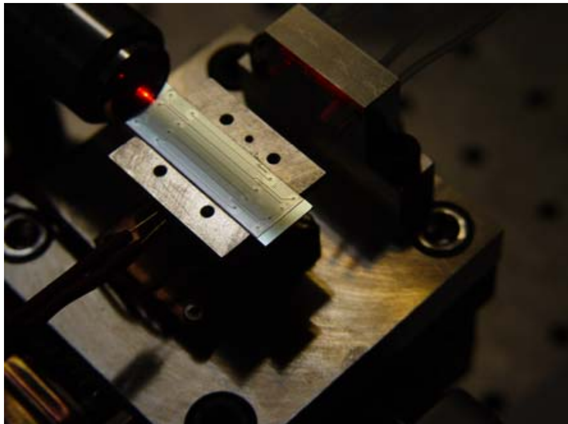




3D-microfluidic network



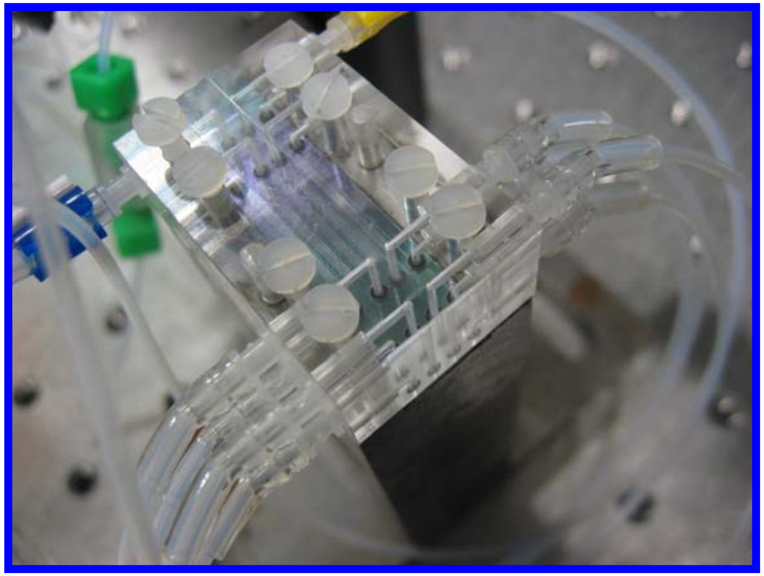
Microfluidic inlet and the embedded microchannel on top the MZI devices



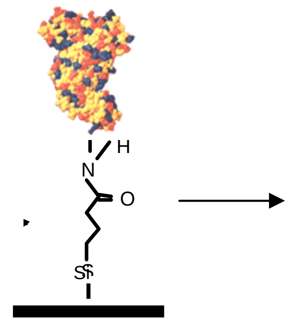
SPIE Photonics West, 2007, 647
J. of Micromechanics and Microengineering, 16, 1006, 2006

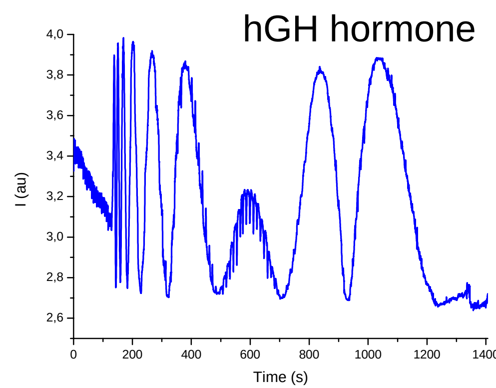
Nanosensor/microfluidics integration

Connection macro/micro



- Modular technology using microfabricated PMMA housing external module
- Easy replacement of the external connections and packaging





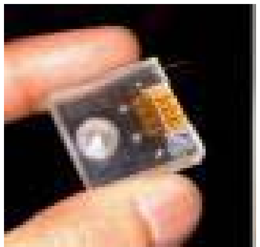
•Steady-state flow rates: 1-1000 µL/min

•Up to 10 Bar without any liquid leakage

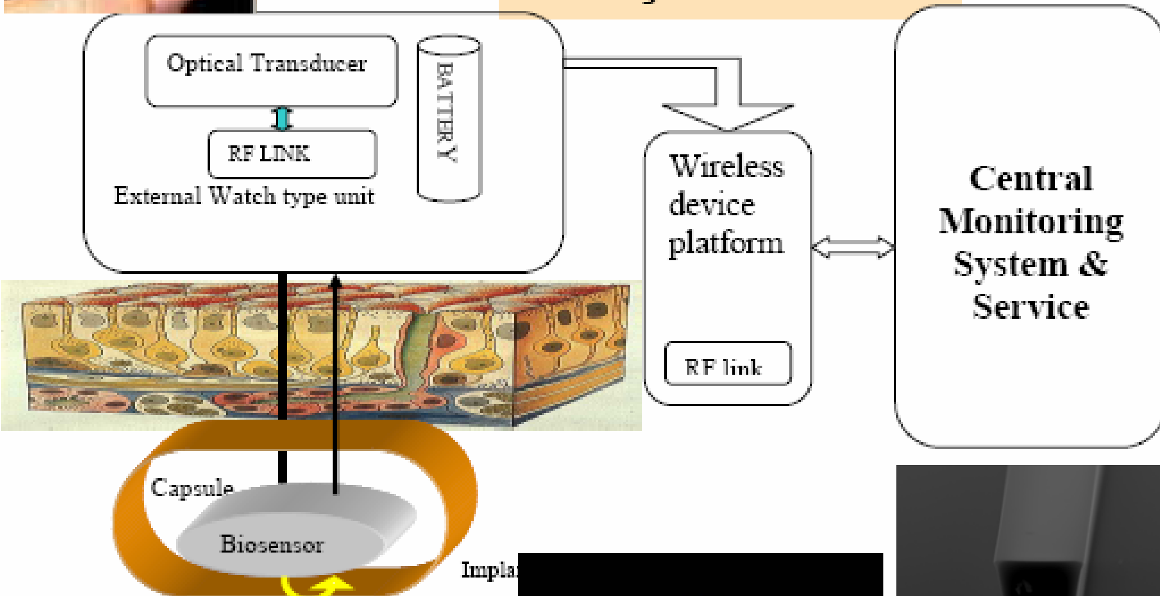
• Reduction of 100 times the chemical reagents consumption!!!

• SU-8 microfluidics is resistant to acid and organic solvents (cleaning and regeneration) www.p-cezanne.com

Development of an implantable biosensor for continuous care and monitoring of diabetic patients (P. CEZANNE)

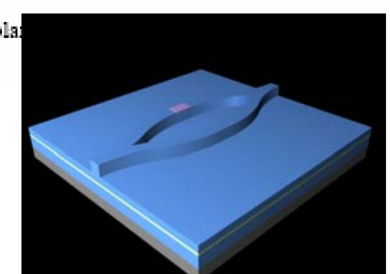


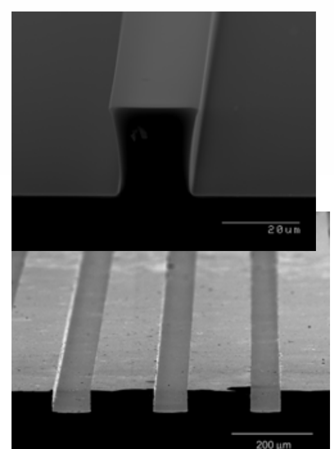
The through-the-skin version



The biosensor will be implanted in the human body during 6 months-1 year, working in a continuous way and with a wireless transmitting data capabilities.

CLALIT HEALTH	ISRAEL
PROTECH AF LTD	UK
LABMAN AUTOMATION	UK
MICROTECH S.R.L.	ITALY
AFCON IND. LTD	ISRAEL
FIMI S.R.L.	ITALY
SIVECO ROMANIA SA	ROMANIA
ROBERT BOSCH GMBH	GE
CNM-CSIC	SPAIN
FRAUNHOFER E.V.	GE





CENTRE D'INVESTIGACIÓ EN NANOCIÈNCIA I NANOTECNOLOGIA

CAMPUS UAB. BELLATERRA. BARCELONA

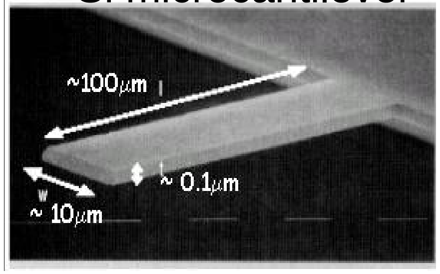
ICN Institut Català de Nanotecnologia

CSIC

NANOMECHANICAL BIOSENSORS

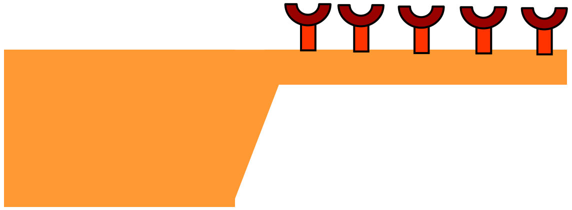
What is a microcantilever sensor?

Si microcantilever

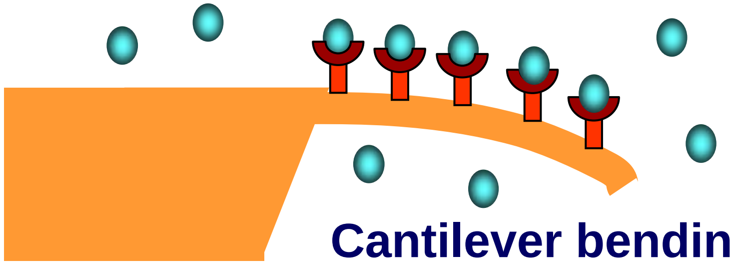


A new class of highly sensitive, label-free and direct biosensor which transduces the molecular recognition of biomolecules into a nanomechanical motion (SNPs-DNA and femtomolar-proteins)

1) Microcantilever functionalization



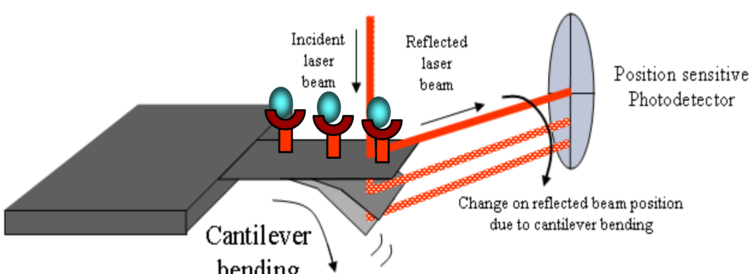
2) Biomolecular Recognition produces a bending of the cantilever due to change of surface stress

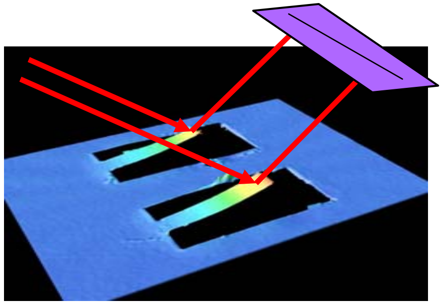


Cantilever bending ~ nm

$$z \approx 4\frac{1-\nu}{Et^2}L^2(\Delta\sigma_t-\Delta\sigma_b)$$

OPTICAL READ-OUT: High sensitivity

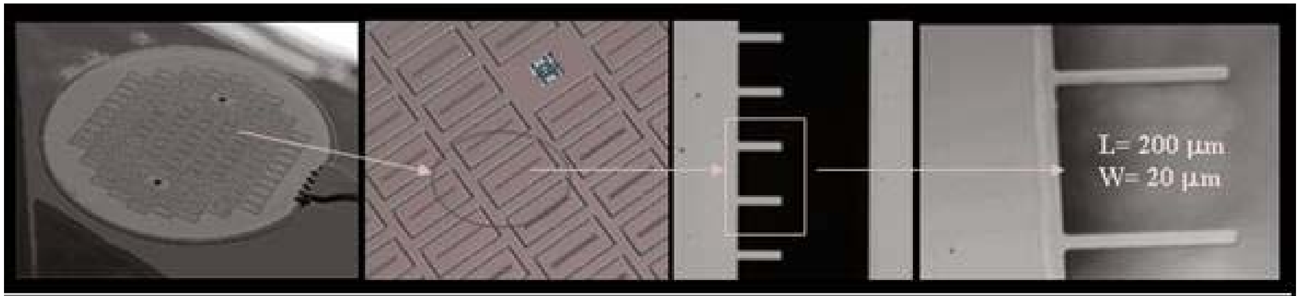




Array of cantilevers

Trends in Anal. Chem (TRAC), 25(3), 196, 2006
Appl. Phys. Lett. 89 (2006) 094109
Cantilever Biosensors In “Optical Biosensors: Today and Tomorrow”
Editorial (si libro): Ed. F. Liegler and C. Taitt. Elsevier, Amsterdam (NL) 2008

Nanomechanical Biosensors



Si cantilevers, 2560 cantilevers, Array of 20-cantilevers, 0.334 mm thick, k= $5.5.10^{-3}$ N/m

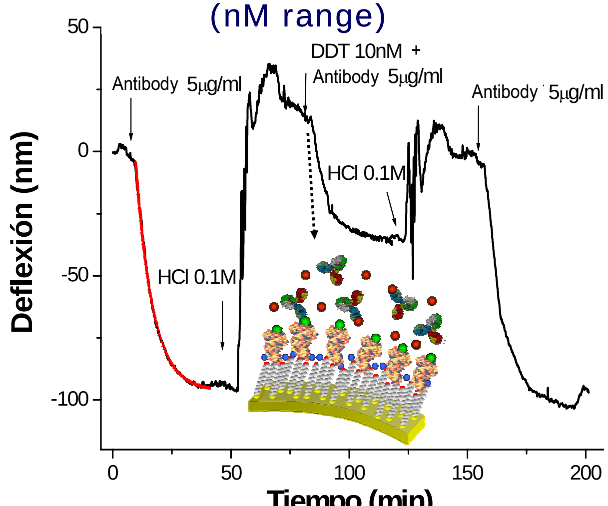
Six time more sensitive for biosensing than commercial ones

minimum detectable deflection 0.1 nm

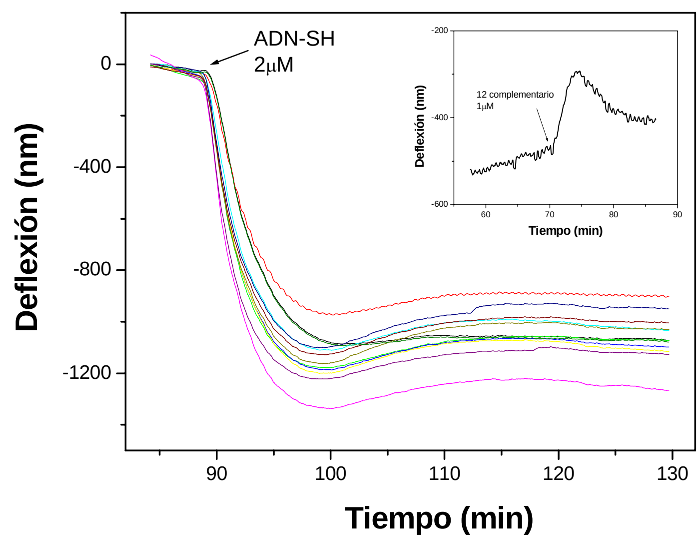
sensitivity expressed in *fm/Hz*$^{-1/2}$: 100

PROTEIN EVALUATION

(nM range)



DNA EVALUATION



CIN2 CENTRE D'INVESTIGACIÓ EN NANOCIÈNCIA I NANOTECNOLOGIA CAMPUS UAB. BELLATERRA. BARCELONA

Langmuir, 20, 9663, 2004

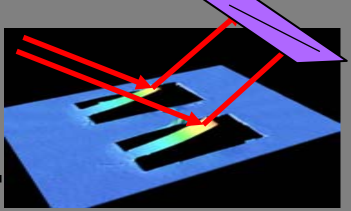
Biosensors & Bioelectronics, 18, 649, 2003

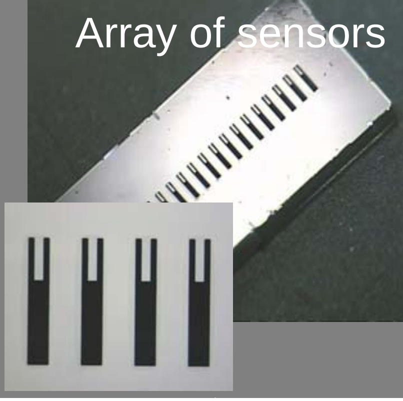
ICN Institut Català de Nanotecnologia

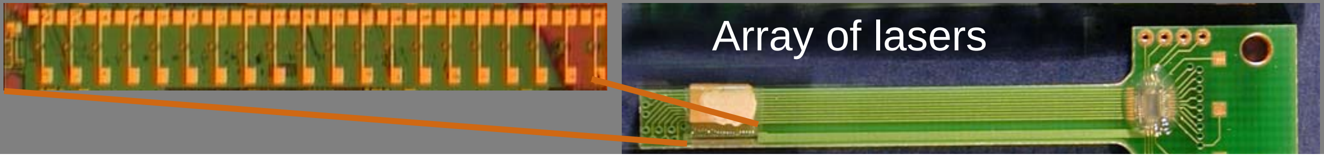
CSIC

Integration in Microsystems

For measuring arrays of microcantilevers.....







▪**Advantages**

✓ High sensitivity

✓ Sub-angstrom resolution

▪**Disadvantages**

✓ Complex integration (difficult alignment): Difficult read-out of arrays

✓ Sequential switching of the laser due to overlapping of the reflection beams

✓ Complex detection by the PD chip

✓ Microlens array onto laser chip

✓ Immobilisation in each cantilever using the 20-flow cell

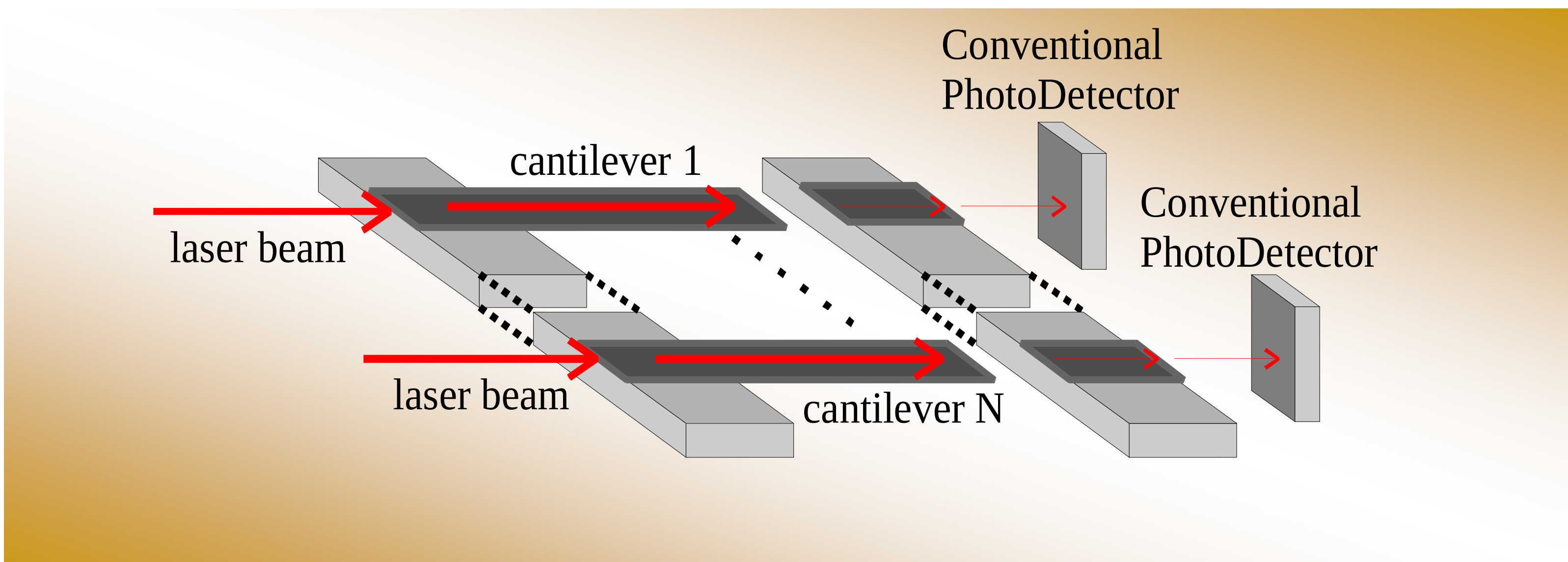
Flow cell-20-channels

- Optics:20-VSCEL+Drivers
- Photodetector array. CMOS circuitry
- Microarrays of 20 microcantilevers
- Microfluidics:flow cell with 20 channels

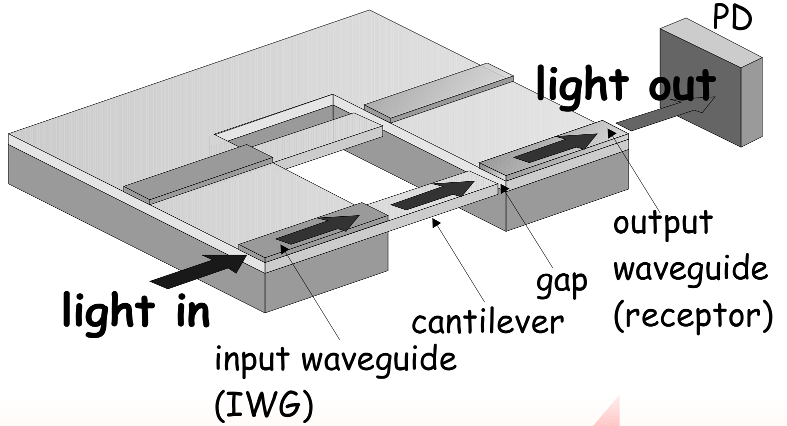
Sensors and Actuators B, 118, 2, 2006

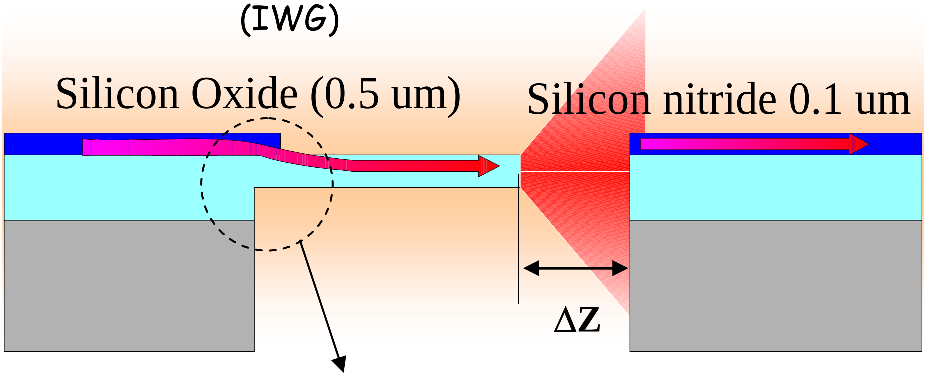
Novel optical waveguide microcantilever sensor

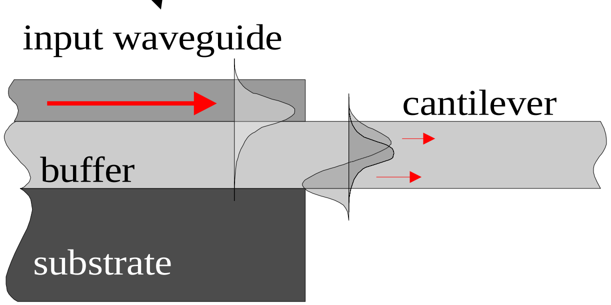
- No alignment (except for light coupling)
- More integrated approach
- No further adjustment
- Conventional photodetectors



OWC: Design and fabrication



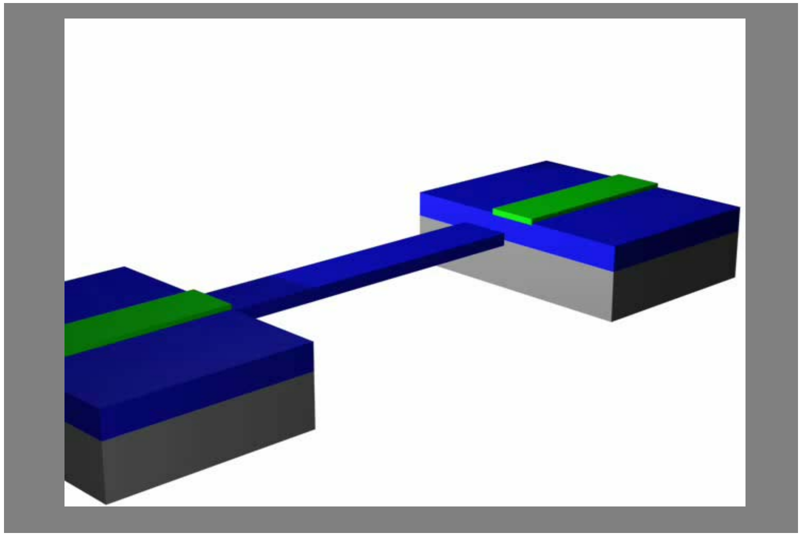




Tested materials:

LPCVD Si_3N_4, PECVD Si_3N_4, PECVD SiO_2, <u>Thermal SiO_2</u>

Silicon oxide cantilever, 600 nm
Silicon nitride receptor, 120 nm
Wavelength 632.8 nm
Gap: 3 µm
2 propagation modes



Coupling efficiency at the junction: up to 70 %

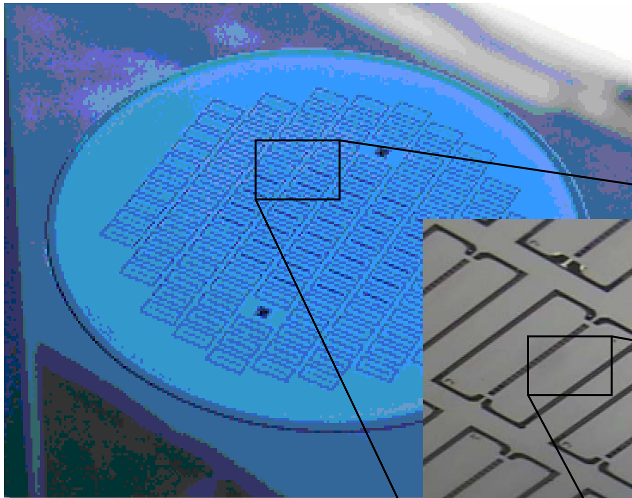
CENTRE D'INVESTIGACIÓ EN NANOCIÈNCIA I NANOTECNOLOGIA
CAMPUS UAB. BELLATERRA. BARCELONA

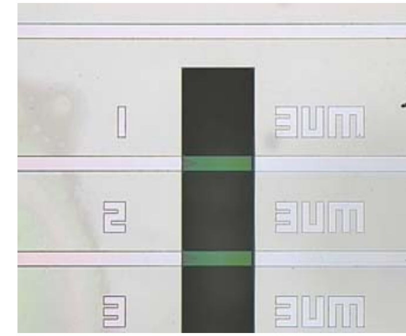
Trends in Anal. Chem (TRAC), 25(3), 196, 2006

Institut Català de Nanotecnologia

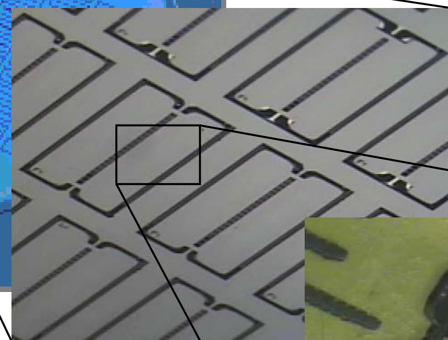
CSIC

OWC: Devices

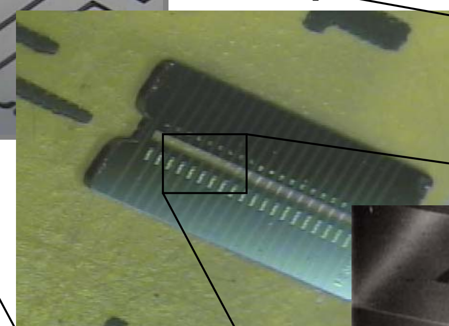




The chips on wafer



A separated chip



The output waveguides

- 2500 cantilevers per wafer
- 20 cantilevers per chip
- Cantilevers are 200 x 40 x 0.6 µm
- direct incoupling
- Spring constant 0.05 N/m

The input waveguides

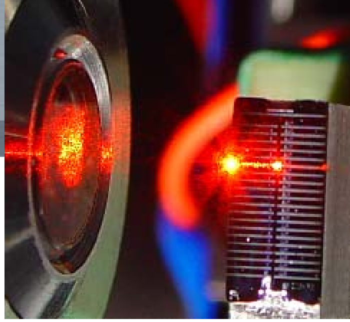
The cavity and cantilevers

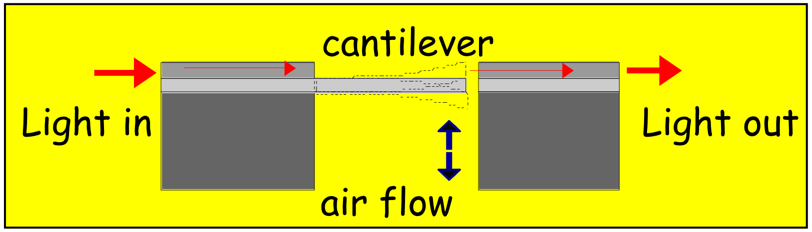
CIN2 CENTRE D'INVESTIGACIÓ EN NANOCIÈNCIA I NANOTECNOLOGIA CAMPUS UAB. BELLATERRA. BARCELONA

Applied Optics, 45(2), 1, 2006
Applied Physics Letters, 92, 011908 2008

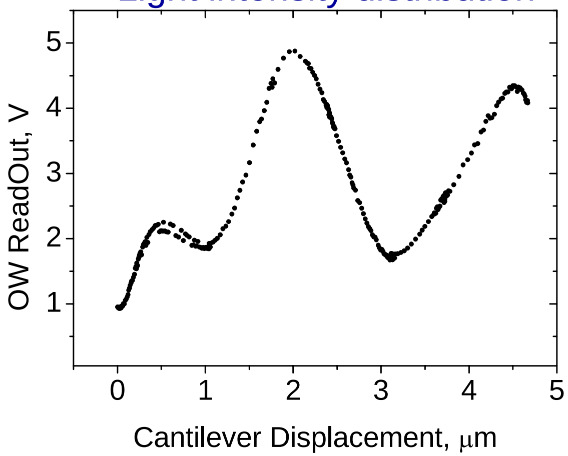
ICN Institut Català de Nanotecnologia CSIC

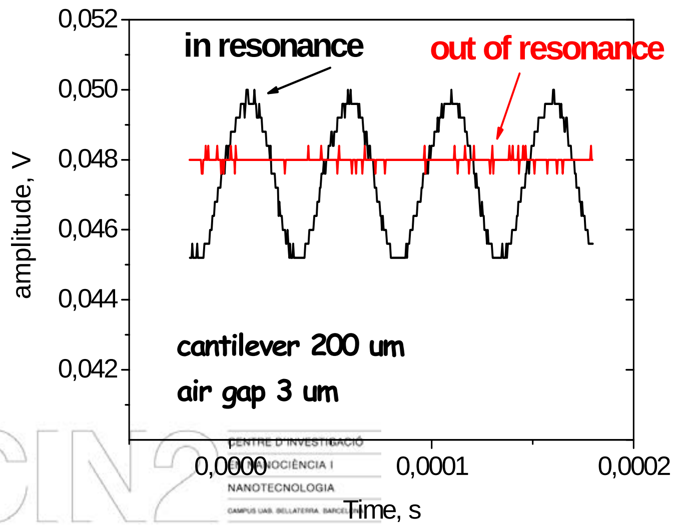
OWC: Performance





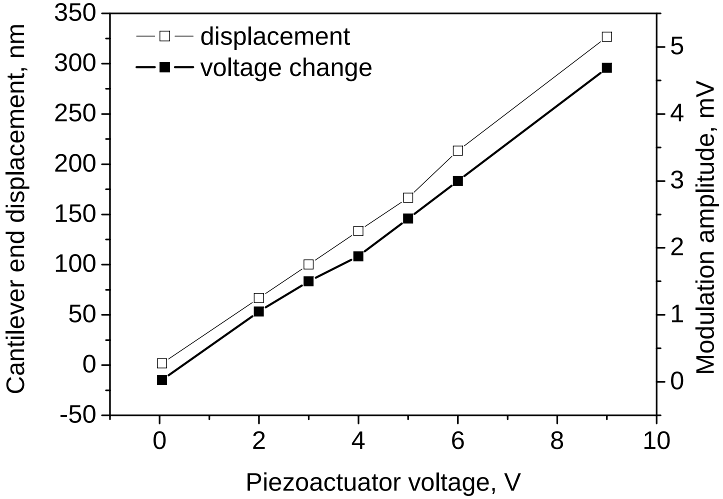
Light intensity distribution





✓OWC chips tested in air

✓operated in dynamic mode



Applying an excitation V of variable frequency and amplitude

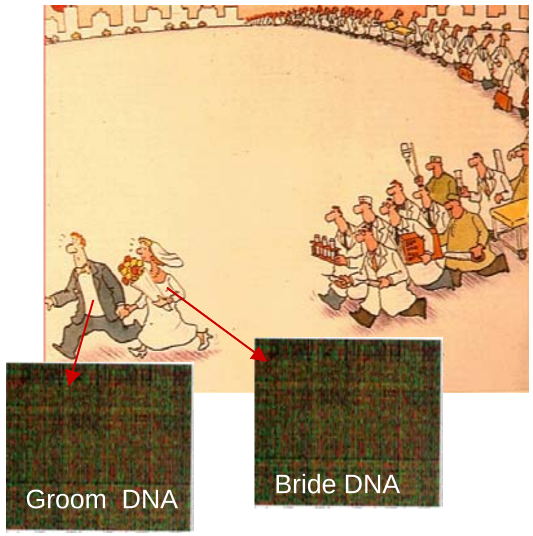
The cantilever displacement can be detected with resolution of **0.04 nm**

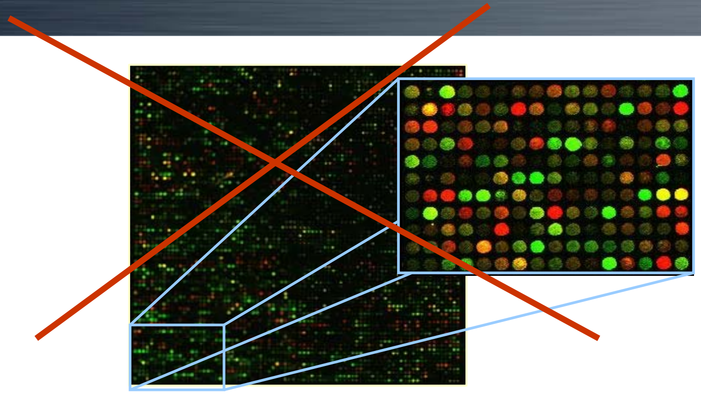
HIGH SENSITIVITY, GOOD PERFORMANCES FOR BIOSENSING

J. Lightwave Tech., 24(5), 2006

Future opportunities

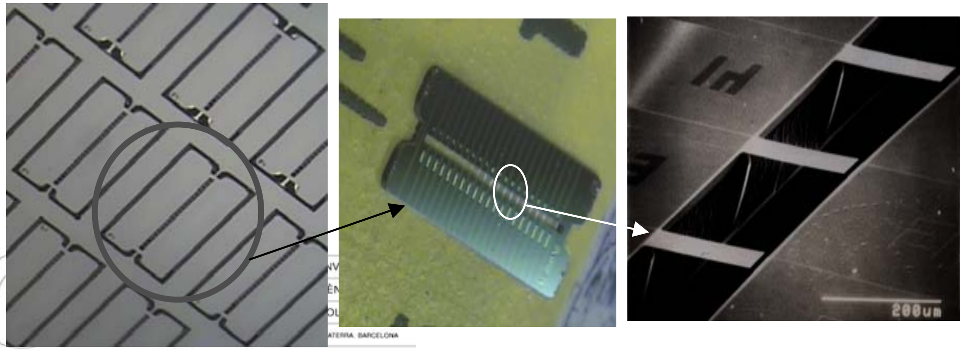
Actual DNA/Protein Biochips





- Need of high amounts of sample: (time consuming, expensive)
- Non-specific interactions
- Indirect read-out: fluorescent labeling. Labels restric assay types
- Low sensitivity for single mismatch detection
- Inconsistent activity of immobilised proteins

"Lab-on-a-chip" Nanobiosensors as an advanced platform

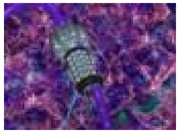


Array of microcantilevers

- Drastic reduction of sample amount
- **Direct read-out**: real time analysis
- High sensitivity
- Micro/nanotechnology: mass production with low cost

In the future......

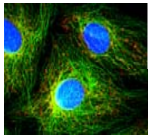
Nanobiosensores en urgencias



Tecnologías
Nanofotónica
Nanotubos, nanopartículas
Nanomecánica, NEMS

Beneficios
Datos en tiempo real e in-situ
Imagen a nivel celular
Herramientas quirúrgicas de precisión guiadas por sensores

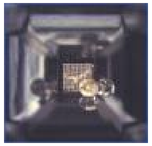
Nanobiosensores en la consulta

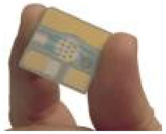


Tecnologías
Biochips
Nanoarrays de alta densidad

Beneficios
Análisis completo en minutos
Diagnósticos rápidos y precisos
Tratamientos específicos y personalizados

Nanobiosensores en casa





Tecnologías
Wireless
Dispositivos portátiles con batería
Displays de alta resolución

Beneficios
Auto-Pruebas diagnósticas simples
Transmisión automática de datos a historial clínico

Acknowledgements

- **Nanobiosensors Group (CIN2-CSIC)**

…, chemist, electronics engineers, molecular biologist,
…ophysics, experts in optoelectronics technology,

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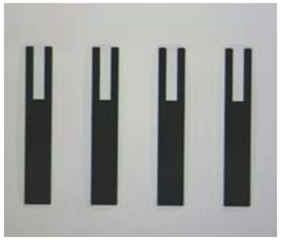
Spanish Ministry of Science
Spanish Ministry of Health (CIBER)
Spanish National Research Council
M. Botín Foundation

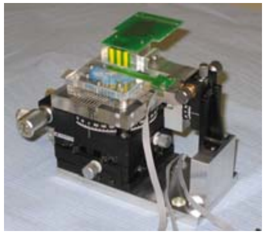


Barcelona, Spain

Nanobiosensors Group: our activity

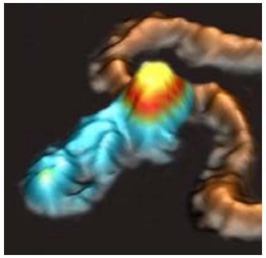
- SPR, Magneto-SPR and LSPR
- Integrated optics Nanophotonic biosensor (MZI)
- µ-Fluidics integration
- Nanomechanical biosensors (standard and optical)
- Carbon Nanotubes Biosensors
- Biofuncionalization
- Lab-on-a-chip technological platforms (in-vitro and in-vivo)
- Applications: environmental control, clinical diagnostics
- Single-molecule biophysics using Magnetic tweezers and AFM

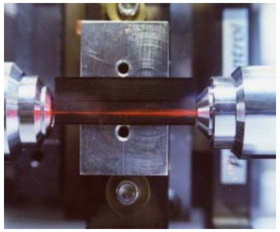


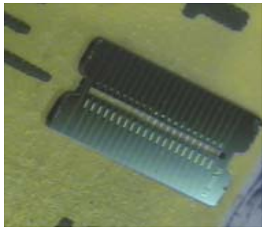


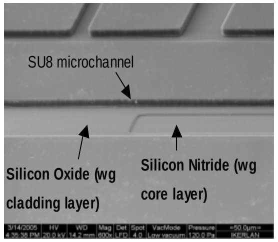
MORE INFO IN

www.cin2.es/biosensores









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with the participation of MONDRAGON COORPERATION (MCC)

NEW SPR VERSION AVAILABLE ON JUNE´08

www.sensia.es